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No. 1

PALMAR SKIN RESISTANCE AS A MEASURE OF PHYSICAL FITNESS

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During past years research in the field of palmar skin resistance (PSR) has frequently been discussed under the inclusive heading of psychogalvanic phenomena. As used in this paper, PSR may be thought of as the impedance offered by the body to the passage of direct current from an external source, the potential difference being applied from the palm of one hand to the palm of the other.

The early literature on electrical phenomena of the skin is amply covered by Landis and DeWick (1929) and Landis (1932), who list 563 titles. Changes in electrical properties of the skin in relation to various probable causes are discussed by many authors: emotional stimuli, Starch (1910); vasodilatation, Aveling and McDowall (1925), Richter (1929a), Darrow (1929); autonomic nervous system and sweat glands, Waller (1919), Richter (1927, 1929a, 1929b), Jeffress (1928), Ryan and Ranseen (1944a, 1944b); reaction to muscular fatigue, Starch (1910), Strauss (1910), Ryan and Ranseen (1944b).

The present study was conducted because of a recent suggestion that the Army Air Forces use PSR changes as a measure of physical fitness. The problem was approached from the standpoint of determining the reliability of PSR measurements and the validity of PSR as an index of physical fitness. Aviation students, averaging 20.7 years of age, were used as experimental subjects. PSR data were secured while the subjects were at rest or while participating in certain kinds of physical activity. Pulse rate and skin temperature were obtained during certain parts of the study for comparison with PSR.

Method. The subject placed each hand, palm downward, in a glass tray filled to a depth of approximately six mm. with an electrolyte (1 per cent aqueous sodium chloride). A layer of cotton gauze on the bottom of the tray prevented the palms from coming in contact with the glass. The trays were mounted upon stools 20 inches in height for the parts of the study involving no exercise, and

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during those parts in which knee bends were used for exercise. When treadmill running was used as a form of physical activity, the trays were mounted upon pedestals 40 inches in height. The electrolyte was used at room temperature (68° – 75° F.) or maintained at $98^{\circ} \pm 3^{\circ}$ F. by means of heating coils placed under the trays. During experimentation, subjects held their hands in the same relative position in the trays, and were requested to exert as nearly uniform pressure on the hands as possible.

It has been shown that PSR changes are in reality changes in electrical reactance within the body which disappear at high frequencies (Waller, 1919; McClendon and Hemingway, 1930). It was considered advisable, therefore, to use direct current in all of the experiments in order to demonstrate PSR changes. The current passing through the subject was constant at all times (0.5 ma.). Connection was made from the current source to the salt solution in which the subject's palms were immersed through two opposed, non-polarizable, copper electrodes via potassium chloride-agar bridges. A galvanometer (Rubicon type 3400H), in parallel with the subject, served as an ohmmeter reading in arbitrary units. Each day the galvanometer scale was calibrated against a standard decade resistance box (General Radio type 602J) so that the readings could be expressed in ohms if desired.

Pulse rate readings were secured with the aid of a Guillemin electrocardio-tachometer. The skin temperature was measured by means of a copper-advance thermocouple attached with adhesive tape to the back of one of the hands of the subject. The temperature of the electrolyte was measured by other thermocouples immersed in the electrolyte in each tray. A selector switch placed in the circuit made possible the use of a single galvanometer (Leeds and Northrup type R) for measuring both skin and electrolyte temperatures.

It was necessary to select physical activities that would be sufficiently vigorous, yet which could be performed while the subject held his hands in the electrolyte. Two types of treadmill running were used: six miles per hour on a horizontal grade, and seven miles per hour with a ten per cent grade. The knee-bend exercise, done at the rate of 44 per minute with the cadence set by a metronome, was also selected as a form of activity. In this exercise the subject when standing bent forward sufficiently to place his hands in the trays. Starting with his legs straight, he lowered his body as far as possible by bending his knees while raising his heels from the floor. He then returned to the starting position. A complete cycle was considered as one knee bend.

Throughout this study the total time during which a subject was being tested, whether at rest or while exercising, is referred to as a testing period. Testing periods were divided into intervals of rest or exercise; these are termed "exercise intervals" or "rest intervals," respectively. For the purpose of becoming accustomed to the electrolyte, at the start of each testing period the subject was required to sit quietly with his hands in the electrolyte for five minutes. This is referred to as the "accommodation interval." A rest interval followed each exercise interval. During each part of the study, the subjects were given identical verbal instructions.

The data. The data were collected in four series each of which is designated numerically. Each experiment in each series is likewise given a numerical designation. Series I, composed of two experiments, and series II, made up of four experiments, were conducted for the purpose of determining reliability of PSR as a measure of physical fitness. Series III and IV were composed of one experiment each. They were done in an attempt to determine the validity of PSR as a measure of physical fitness.

Treatment of data. The original PSR readings were transcribed in arbitrary units directly from the galvanometer scale. The frequency distribution of single records from 208 different subjects did not satisfy statistical tests for normality: though the distribution was satisfactorily mesokurtic, it was skewed to the right. When the scale readings, R , were transformed to logarithms, $100 \log_{10} R$, the tests for normality were satisfied (table 1). These logarithmic units are referred to in this paper as skin resistance units (SRU). The more SRU, the higher the palmar skin resistance.

In the analysis of the data for test reliability, the method of the analysis of variance was used as suggested by Hoyt (1941) and Jackson (1942). The relia-

TABLE 1
Test for normality of the distribution of PSR; galvanometer scale readings (R) and ($100 \log_{10} R$). $N = 208$

UNIT	MEAN	S.D.	SKEWNESS			KURTOSIS		
			g_1	s_1	t_1	g_2	s_2	t_2
(R)	49.8	21.2	+0.820	± 0.169	4.852*	+0.173	± 0.336	0.516
($100 \log_{10} R$)	164.9	18.6	-0.182	± 0.169	1.077	-0.311	± 0.336	0.925

* Significantly large.

bility coefficient r_{tt} indicates what fraction of the subject variance in the data is due to actual differences between subjects and not to uncontrolled error. Thus, if $r_{tt} = 0.98$, only 2 per cent of the subject variance may be ascribed to sources of "error," including interactions. When subject differences alone contribute so much to variation in the results of a test, the test is said to be highly reliable.

Reliability. The reliability of PSR measurements was evaluated during periods in which the subjects were at rest and during periods of alternate rest and exercise, with the electrolyte at room temperature or at 98°F. In series I the variation of PSR was observed with the subjects at rest. In one experiment (expt. 1), determinations were made every 30 seconds upon 29 subjects, during four two-minute intervals in which the hands were held in the electrolyte, alternating with similar intervals with the hands out of the electrolyte. Twenty determinations each of PSR, pulse rate, and skin temperature were secured for each subject. Average SRU values for the 29 subjects are shown in table 2. The table shows an average increase in SRU from the first through the fifth determination. Table 10 (A) shows that variances from all indicated sources are

significantly larger than that assigned to residual experimental "error"; further, not only is the variation ascribed to intervals and determinations greater than that expected on the basis of chance, but differences between subjects account for all but 0.2 per cent of the remaining variance (the reliability, $r_u = 0.998$). The curves in figure 1 represent means for 29 individuals.

TABLE 2

Means in skin resistance units secured during four two-minute intervals with subjects at rest—five determinations during each interval—30 seconds between determinations*

INTERVALS	DETERMINATIONS WITHIN INTERVALS					AVERAGE
	1	2	3	4	5	
1	167.8	168.9	170.8	172.0	172.8	170.4
2	169.2	170.3	171.6	172.8	173.4	171.5
3	171.4	172.0	173.0	172.8	173.6	172.6
4	169.0	169.9	171.2	172.1	172.9	171.0
Average..	169.3	170.3	171.7	172.4	173.2	171.4 grand mean (= 5230 ohms)

* Means in the following tables are shown in skin resistance units.

TABLE 3

Means for determinations secured every 30 seconds during a five-minute interval of rest on each of four days with at least one day between testing periods

DETERMINATIONS WITHIN INTERVALS	DAYS				AVERAGE
	1	2	3	4	
1	147.9	177.2	178.4	177.0	176.9
2	175.2	176.9	178.7	177.0	177.0
3	174.8	177.3	178.0	177.1	176.8
4	174.8	177.4	178.0	176.4	176.6
5	174.5	177.1	177.8	175.8	176.3
6	174.8	176.1	178.0	175.5	176.1
7	175.0	176.4	178.4	176.0	176.5
8	175.2	176.0	178.6	176.2	176.5
9	175.1	175.8	178.7	176.0	176.4
10	175.5	175.7	179.1	176.3	176.6
Average...	175.0	176.6	178.4	176.3	176.6 grand mean (= 4100 ohms)

In experiment 2, series I, 29 (other) subjects were each tested while at rest during four testing periods, with at least one day between periods. Ten determinations were secured for each subject in this manner during each testing period. Averages appear in table 3. The method of analysis of variance yielded the high reliability coefficient, $r_u = 0.991$ (table 10 (B)).

In series II, the reliability of PSR was tested under conditions of alternating

exercise and rest intervals. Two experiments were conducted using the knee-bend exercise and two using the treadmill run at seven miles per hour with a ten per cent grade. Knee-bend experiments were performed as follows: there were two one-minute intervals of exercise, each of which was followed by two minutes of rest; then, two two-minute exercise intervals, followed by five minutes of rest.

TABLE 4

Means for determinations made during two one-minute exercise intervals and two two-minute exercise intervals—determinations made every 30 seconds—(A) electrolyte room temperature; (B) electrolyte 98°F.

(A) ONE-MINUTE INTERVALS	DETERMINATIONS WITHIN INTERVALS					AVERAGE
	1	2	3	4	5	
1	171.8	159.5	155.5			162.2
2	158.7	155.8	154.2			156.2
Average . .	165.3	157.6	154.8			159.2 grand mean (= 2940 ohms)
TWO-MINUTE INTERVALS						
1	157.2	154.0	152.0	151.4	151.4	153.2
2	155.2	151.4	149.9	149.8	150.3	151.3
Average . .	156.2	152.7	150.9	150.6	150.8	152.3 grand mean (= 2570 ohms)
(B) ONE-MINUTE INTERVALS						
1	175.5	169.3	168.1			171.0
2	169.9	168.3	167.9			168.7
Average . .	172.7	168.8	168.0			169.8 grand mean (= 3500 ohms)
TWO-MINUTE INTERVALS						
1	169.8	168.0	166.3	165.9	166.6	167.3
2	168.9	167.0	166.5	166.4	166.7	167.0
Average . .	169.3	167.5	166.4	166.1	166.7	167.2 grand mean (= 3300 ohms)

In the treadmill run, there were two two-minute exercise intervals, each followed by a five-minute rest interval. For each exercise, trials were made with the electrolyte at room temperature and at 98°F., so that there were four experimental conditions in which the 144 subjects were divided into four groups. Average readings of PSR for knee-bends are shown in table 4. The means for

the treadmill run are contained in table 5. Figure 2 shows PSR, skin temperature, and pulse rate for the knee-bend exercise, while figure 3 shows comparable data for the treadmill run.

Variance data for series II are shown in table 10. The analysis for knee-bends

TABLE 5

*Means for determinations made every 30 seconds during two two-minute exercise intervals—
(A) electrolyte at room temperature; (B) electrolyte at 98°F.*

(A) INTERVALS	DETERMINATIONS WITHIN INTERVALS					AVERAGE
	1	2	3	4	5	
1	166.7	138.5	137.7	140.2	147.5	146.1
2	164.3	143.1	143.4	144.4	150.3	149.1
Average..	165.5	140.8	140.6	142.3	148.9	147.6 grand mean (= 2500 ohms)
(B) INTERVALS						
	1	2	3	4	5	
1	165.3	152.5	153.0	153.3	158.1	156.4
2	165.4	154.3	154.0	154.6	158.9	157.5
Average..	165.3	153.4	153.5	153.9	158.5	156.9 grand mean (= 2680 ohms)

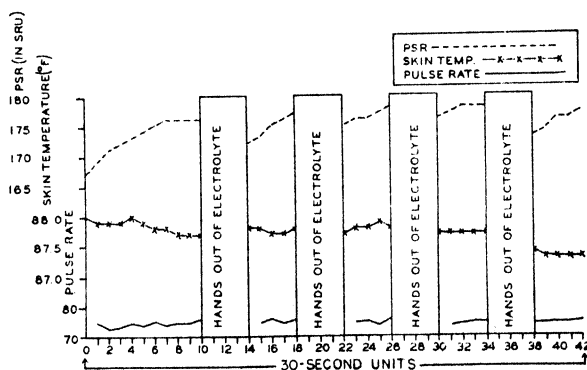


Fig. 1. Effect on PSR, pulse rate, and skin temperature of alternately holding hands in and removing them from electrolyte while subjects were at rest. Electrolyte at room temperature.

appears in 10 (C) (electrolyte room temperature), and in 10 (D) (electrolyte 98°F.). The data for the treadmill run are shown in 10 (E) (electrolyte room temperature) and 10 (F) (electrolyte 98°F.). The coefficients of reliability (r_u) derived from these analyses of variance were uniformly high: Knee bends, room temperature (one-minute exercise interval), 0.920; knee bends, room

temperature (two-minute exercise interval), 0.992; knee bends, 98°F. (one-minute exercise interval), 0.977; knee bends, 98°F. (two-minute exercise interval), 0.998; treadmill run, room temperature, 0.988; treadmill run, 98°F., 0.991.

The effect of variation of the temperature of the electrolyte upon PSR is evident on inspection of the data. Comparison of the grand mean PSR values at the two temperatures for both types of exercise reveals that PSR readings obtained at 98°F. are higher than those obtained at room temperature (table 6).

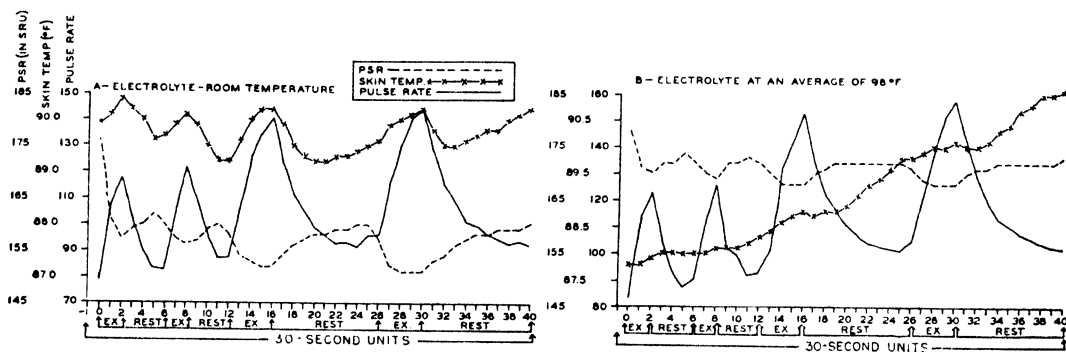


Fig. 2. Effect of knee bends upon PSR, pulse rate, and skin temperature. Electrolyte at room temperature and at an average of 98°F.

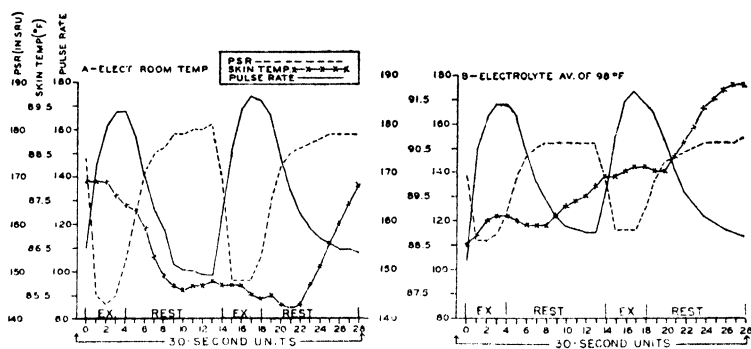


Fig. 3. Effect of treadmill run (7 m.p.h., 10 per cent grade) upon PSR, pulse rate, and skin temperature. Electrolyte at room temperature and at an average of 98°F.

The high values of t indicate that this difference is so large that it would seldom occur by chance. Because of the demonstrated effect of temperature on PSR, subsequent experiments were conducted with the electrolyte at 98°F., rather than at the more variable room temperature.

Validity. Having established that PSR shows a high reliability from interval to interval and from test period to test period during rest and exercise, while the electrolyte is either at room temperature or maintained at an average of 98°F., it was considered necessary to determine the validity of PSR as a measure of

physical fitness. This part of the study was based upon the hypothesis that fatiguing physical activity such as the treadmill run causes physiological changes which vary with the intensity and duration of exercise. It is further assumed that the PSR responses of all individuals tested under the same conditions should be in the same relative direction; i.e., the participation in a treadmill run ten

TABLE 6

Difference between grand means for knee bends and treadmill run with electrolyte at room temperature and at an average of 98°F.

	KNEE BENDS		TREADMILL
	1 min. interval	2 min. interval	
(Room temperature).....	159.2	152.3	147.6
(Average 98°F.).....	169.8	167.2	156.9
Difference.....	10.6	14.9	9.3
Standard error (computed from error variance including interactions).....	±0.56	±0.15	±0.44
t.....	18.9	99.0	21.0

TABLE 7

Changes in mean skin resistance units resulting from two, four, six, eight, and ten minute intervals of running with at least one day between intervals—determinations made before and after exercise

	DURATION OF EXERCISE IN MINUTES					AVERAGE
	2	4	6	8	10	
Changes in SRU.....	2.18	2.72	3.56	4.88	5.48	3.76

TABLE 8

Analysis of variance applied to regression of change in skin resistance units

SOURCE OF VARIANCE	df	MEAN SQUARE
Subjects, S.....	49	90.324*
Regression, R.....	1	383.688*
S × R.....	49	18.729*
Error.....	150	8.979
Total.....	249	

* Mean square is significantly large compared with "error" term.

minutes in duration would cause either a consistent rise or a consistent fall for all individuals.

In series III, each of 50 subjects was required to run on the treadmill at the rate of six miles per hour (horizontal grade) for testing periods involving intervals of two, four, six, eight and ten minutes, with at least one day between testing

periods. In order to eliminate error which might have occurred by following a definite sequence of testing order as to the length of the exercise interval,

TABLE 9

Correlation between duration of treadmill run to exhaustion with the before-exercise skin resistance and with changes in skin resistance during run

	MEAN	S.D.	r
Duration of running (minutes).....	20.06	9.909	
Initial SRU.....	150.825	10.565	+0.066
Change in SRU.....	14.943	10.397	+0.209

TABLE 10

Analysis of variance of PSR data

SOURCE OF VARIANCE	(A)		(B)		(C) (1 MIN. INT.)		(C) (2 MIN. INT.)	
	df	Mean square	df	Mean square	df	Mean square	df	Mean square
Subjects, S.....	28	7,908.37*	28	5,074.41*	34	596.28*	34	619.41*
Intervals, I.....	3	114.97*	3	570.37*	1	1,880.10*	1	305.40*
Determinations, D.....	4	281.95*	9	8.51*	2	2,036.05*	4	389.60*
Interactions:								
S × I.....	84	98.49*	84	480.05*	34	85.26*	34	14.09*
S × D.....	112	11.95*	252	10.97	68	3.08	136	5.88*
I × D.....	12	8.81*	27	6.75	2	673.95*	4	4.90*
Error.....	336	2.09	756	9.34	68	24.02	136	1.94
Total.....	579		1,159		209		349	

SOURCE OF VARIANCE	(D) (1 MIN. INT.)		(D) (2 MIN. INT.)		(E)		(F)	
	df	Mean square	df	Mean square	df	Mean Square	df	Mean square
Subjects, S.....	34	700.6*	34	1,531.9*	38	4,053.30*	34	2,848.5*
Intervals, I.....	1	269.7*	1	3.1	1	868.52*	1	92.6*
Determinations, D.....	2	436.9*	4	118.2*	4	8,699.49*	4	1,858.1*
Interactions:								
S × I.....	34	32.6*	34	5.9*	38	53.93*	34	36.4*
S × D.....	68	16.3	136	3.0*	152	77.70*	136	46.4*
I × D.....	2	144.1*	4	8.0*	4	200.10*	4	6.9*
Error.....	68	10.2	136	1.2	152	12.12	136	5.9
Total.....	209		349		389		349	

Note: *df* = degrees of freedom.

* = Mean square is significantly large compared with "error" term.

a randomization table was constructed and a separate sequence of testing was followed for each subject. PSR determinations were made at the start and at the conclusion of exercise.

The subject stood up 20 seconds before the start of exercise. The treadmill was started at a slow speed and gradually brought to a speed of six miles per hour. The initial PSR reading was taken at the time of starting the run. The speed of the treadmill was not changed until the final reading was taken: two, four, six, eight or ten minutes after the actual start of exercise. Thus, the two-minute interval involved two minutes of running at six miles per hour, although the subject walked and ran for a few seconds prior to the first reading and ten to fifteen seconds after the final reading.

Table 7 shows that on the average, for each two-minute increase in duration of exercise, there was an increase of 0.88 in SRU difference. However, all individuals did not react in the same manner, some showing significant departures from the average regression line. Thus, the value of the variance for " $S \times R$ " (differential response of individuals to regression) in table 8 is significantly large compared with the "error" term. Based upon these data, it is evident that though the regression of 0.88 SRU difference for each two minutes of exercise is a real one, i.e., significantly different from zero, the data are not consistent enough to warrant high predictability for individuals.

In series IV an attempt was made to determine the validity of PSR as a measure of physical fitness based upon endurance in the treadmill run. Thirty-six subjects each ran on the treadmill at six miles per hour until exhausted. The mean duration was 20.06 minutes, the range being 10 to 55 minutes. PSR, pulse rate, and skin temperature were determined every 30 seconds during the run, and for five-minute intervals before and after the run. The PSR data are shown in table 9. Although obtained at room temperature, they were useful, since it was found that controlling the electrolyte at a temperature of 98°F. merely gave higher and slightly more stable readings.

It will be noted that there was a very low correlation coefficient between the duration of running and the PSR at the start of the run ($r = +0.066$); likewise, there was a very low correlation between duration of running and the SRU change during running ($r = +0.209$). To be significant, r must exceed $+0.42$. In figure 4 the data were divided arbitrarily into two groups; 21 subjects with endurance times ranging from 10 to 17 minutes, inclusive (4A), and ten with endurance times ranging from 18 to 25 minutes, inclusive (4B). Since the inclusion of the records of five subjects running in excess of 25 minutes would have contributed little and would have caused distortion, they were omitted. Visual comparison of figures 4A and 4B reveal no striking differences in form.

Relationship between PSR and other measurements. In parts of the present study data relative to skin temperature and pulse rate were collected simultaneously with PSR data, for the purpose of examining the relationships between the three variables. Figures 1 shows that while the subjects were at rest there was a definite rise in SRU during the five-minute accommodation period and during each interval while the hands were held in the electrolyte. There was a decline of 0.8°F. in skin temperature from the first to the final reading, while the pulse rate remained practically constant throughout. Figures 2 and 3 show that during alternate intervals of rest and knee bends the PSR readings were con-

sistently higher and fluctuated less when the electrolyte was at 98°F. than when it was at room temperature. At both temperatures there was an inverse relationship between PSR and pulse rate, showing an increase in pulse rate during exercise and a decline during rest, with opposite changes in PSR. The steady rise in skin temperature noted in figures 2B and 3B was, without doubt, due to the effect of the warm electrolyte, since the skin temperature thermocouple, though not in the electrolyte itself, was attached to the back of the subject's hand.

During the strenuous exercise of short duration done in series II (figs. 2 and 3) there was a much greater range of reaction in both pulse rate and PSR. Again, in both instances, PSR reacted inversely to pulse rate with but a slight lag in PSR. In figure 3A the greatest variation in PSR occurs during the rise within the rest interval between the first and second intervals of exercise. In both graphs, the highest mean pulse rate was reached during the second interval of

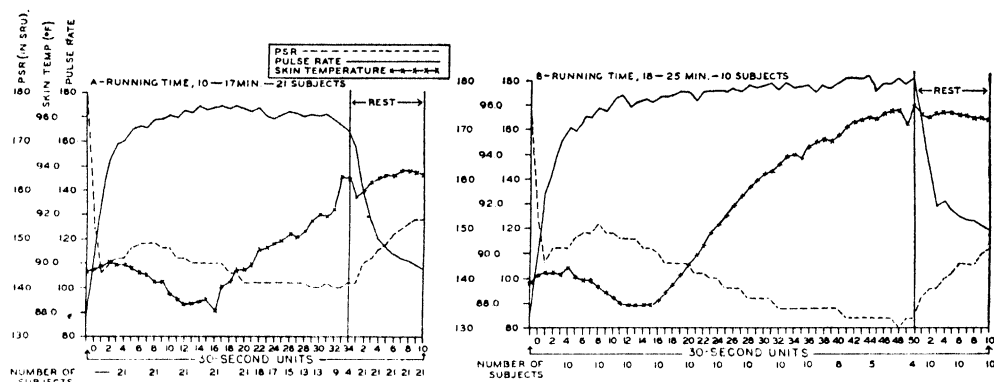


Fig. 4. Effect of treadmill run (6 m.p.h., horizontal grade) upon PSR, pulse rate, and skin temperature. Electrolyte at room temperature.

exercise, the difference between the two mean maxima being 0.6 beat per minute. The skin temperature showed a rapid decline, fluctuating only slightly; it reached a minimum of 85.2°F. after a minute and a half in the final rest period. There followed a rapid rise to 87.8°F. The steady rise shown in figure 3B was again doubtlessly due to the warm electrolyte.

In general the treadmill run for endurance (fig. 4) shows an inverse relationship between PSR and pulse rate. There was an exception in the brief interval between 30 seconds and four minutes after the start of the exercise. During this time there was a slight rise in PSR, followed by a decline which in the main was more consistent than the rise in pulse rate.

The skin temperature for both groups showed a slight increase after the start of exercise followed by a decline, the minimum being reached after about six minutes of exercise. Following this, there was generally a steady increase in skin temperature which continued through the remainder of exercise and four minutes of rest for the 10-to-17-minute group (fig. 4A), and to the conclusion

of exercise for the 18-to-25-minute group (fig. 4B). In general in this part of the study, there is an inverse graphic relationship between skin temperature and PSR.

SUMMARY

A study was conducted in an attempt to determine the reliability of palmar skin resistance (PSR) during rest and exercise, and the validity of PSR as a measure of physical fitness. The reliability of PSR determinations was high ($r_{tt} = 0.920$ to 0.998) during rest and during exercise (knee bends and treadmill run), when testing was accomplished on single days or on successive days.

In spite of its high reliability, PSR is not a good measure of physical fitness; for, based on the treadmill run to exhaustion, there is no correlation between resting PSR and endurance, and there is evidence of poor correlation between PSR and endurance under all conditions studied. PSR is not a valid measure of fatigue: though the majority of subjects showed a decrement in PSR with increased fatigue caused by prolonging the exercise interval, many individuals showed the reverse change.

In general, PSR decreases during exercise and increases during rest. The decrement is more pronounced during intensive than during mild exercise. In the main, PSR shows an inverse relationship to pulse rate. The temperature of the electrolyte in which the hands are immersed influences PSR readings, but has no effect upon the reliability of the test.

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REFERENCES

- AVELING, F. AND R. J. S. McDOWELL. *J. Physiol.* **60**: 316, 1925.
 DARROW, C. W. *Psychol. Bull.* **26**: 155, 1929.
 HOYT, C. *Psychometrika* **6**: 153, 1941.
 JACKSON, R. W. B. *Psychometrika* **3**: 157, 1942.
 JEFFRESS, L. A. *J. Exper. Psychol.* **11**: 130, 1928.
 LANDIS, C. *Psychol. Bull.* **29**: 693, 1932.
 LANDIS, C. AND H. N. DEWICK. *Psychol. Bull.* **26**: 64, 1929.
 MCCLENDON, J. F. AND A. HEMINGWAY. *This Journal* **94**: 77, 1930.
 RICHTER, C. P. *Brain* **50**: 216, 1927.
 Bull. Johns Hopkins Hosp. **45**: 56, 1929a.
 This Journal **88**: 596, 1929b.
 RYAN, A. H. AND E. L. RANSEEN. *Fed. Proc.* **3**: 40, 1944a.
 This Journal **142**: 68, 1944b.
 STARCH, D. *Psychol. Rev.* **17**: 19, 1910.
 STRAUSS, W. *Ztschr. f. Hyg. u. Infektionskrankh.* **107**: 56, 1927.
 WALLER, A. D. *Proc. Roy. Soc. London* **B91**: 17, 1919.

STUDIES ON THE NUTRITIVE VALUE OF LACTOSE AND GALACTOSE WITH THE SINGLE-FOOD CHOICE METHOD¹

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A number of reports have appeared concerning the inadequacy of lactose as a dietary carbohydrate for the rat. Not only has retardation of growth been observed on lactose-containing rations, but a number of additional findings have been reported including diarrhea (1), dilatation of the cecum (1, 2), alopecia (2), and a number of early deaths (2). The present series of experiments were planned to obtain further information on the poor nutritive showing of lactose-containing rations.

METHODS. Female rats of the Sprague-Dawley strain were raised to maturity on a stock ration and were selected for the following experiment at approximately 70 days of age at an average weight of 146 grams (range 134 to 172 grams). The "single food choice" technique as used by Richter, Holt and others (3-5) was employed. Rats of a standard age and weight were fed diets consisting of a single foodstuff, and the length of survival on the various rations determined. Animals were placed in individual cages with screen bottoms to prevent access to feces, and sufficient food was administered to assure *ad lib* feeding. Daily observations were made of food and water intake, and body weight was recorded weekly.

Seventy-two female rats were used in the initial experiment: 12 rats on lactose² alone; 10 rats on galactose;³ 10 rats on dextrose; 10 rats on sucrose; 10 rats on butter fat; and 12 rats on a diet of 50 per cent-dextrose-50 per cent-galactose. A control group of 8 rats had access only to water.

RESULTS. *Survival time.* Table 1 summarizes the individual and average survival times of rats fed the various diets. Survival on lactose or galactose was distinctly inferior to that of other substances tested and differed but slightly from that of fasted controls (a finding in agreement with previously published results (4, 5)). Survival on 50 per cent-dextrose-50 per cent-galactose however was significantly longer than that on lactose alone and was similar to that obtained in the sucrose, dextrose or butter fat series. These findings suggest that the poor nutritive value of lactose as measured by length of survival of lactose-fed rats was due to an inability on the part of the rat to hydrolyze this sugar in adequate amounts under the conditions of the present experiment. This suggestion gains support from the observation that 100 per cent of lactose-fed rats showed at autopsy distention of the large intestine, marked dilatation of

¹ The subject matter of this paper has been undertaken in cooperation with the Quartermaster Corps Committee on Food Research.

² Merck and Co., Rahway, New Jersey.

³ General Biochemicals Corp., Chagrin Falls, Ohio.

the cecum and some degree of diarrhea; none of the animals on other rations were thus affected.

Gross findings. With the exception of a "pot-bellied" appearance in rats of the lactose series, none of the animals showed significant changes with the exception of those on the 50-per-cent-dextrose-50-per-cent-galactose ration. In this latter series with the exception of one animal which died on the 17th day, 100

TABLE 1
Survival time (in days)

DIET	NUMBER OF ANIMALS	INITIAL WEIGHT	SURVIVAL TIME	AVERAGE SURVIVAL TIME
		grams	days	days
Lactose.....	12	141.4	4, 4, 5, 5, 6, 7, 7, 7, 8, 8, 10, 10	6.9
Galactose.....	10	146.5	4, 4, 4, 5, 5, 5, 6, 6, 7, 7	5.3
Dextrose.....	10	139.7	32, 33, 35, 36, 36, 37, 38, 40, 46, 46	37.9
Sucrose.....	10	145.1	26, 31, 31, 33, 33, 33, 38, 38, 41, 49	35.3
Butter fat.....	10	143.8	32, 32, 34, 35, 35, 35, 39, 42, 43, 52	37.9
50%-dextrose				
50%-galactose...	12	144.3	17, 26, 28, 30, 30, 31, 32, 32, 32, 35, 38, 43	31.2
No food.....	8	138.6	4, 4, 4, 5, 5, 5, 6, 6	4.9

TABLE 2
*Food and water intake**

EXPERIMENTAL RATION	NUMBER OF ANIMALS	DAILY FOOD INTAKE PER RAT DURING FOLLOWING WEEKS OF EXPERIMENT				DAILY WATER INTAKE PER RAT DURING FOLLOWING WEEKS OF EXPERIMENT			
		1st	2nd	3rd	4th	1st	2nd	3rd	4th
		cals./day				cc./day			
Lactose.....	12	16				14			
Galactose.....	10	46				77			
Dextrose.....	10	45	32	26	26	8	5	6	4
Sucrose.....	10	42	28	22	21	12	8	6	5
Butter fat.....	10	27	30	31	20	16	9	7	6
50%-dextrose-50%-galactose.....	20	41	62	58	44	49	84	67	45
30%-butter fat-70%-galactose.....	8	38	52	46	42	38	45	40	30

* Calories were computed on the following basis: carbohydrates, 4 calories per gram; fats, 9 calories per gram.

per cent of the rats developed a severe flaccid paralysis of the hind limbs. Irregularity of gait was observed as early as the 16th day, although a severe stage of paralysis did not generally develop before the 26th day. In the more advanced stages animals lost completely use of their hind limbs and could move only by dragging themselves along on their forepaws. In addition to paralysis animals on the 50-per-cent-dextrose-50-per-cent-galactose ration developed mature

cataracts. One hundred per cent of the rats were affected, the average time of onset being 20.5 days (range 17 to 25 days).

Further studies on "galactose paralysis." In view of the work of Schantz et al. (6, 7) that fatty acids of 12 or more even numbered carbon atoms are required for the oxidation of galactose, the following experiment was conducted in an effort to determine the effects of dietary fat on the incidence and severity of "galactose paralysis." The experimental procedure was similar to that employed above. Female rats of the Sprague-Dawley strain were placed on the following rations at approximately 70 days of age at an average weight of 139 grams (range 136 to 165 grams). Twenty-four rats were used in the present experiment consisting of 3 groups of 8 rats each. The following diets were employed: (1) 50-per-cent-dextrose-50-per-cent-galactose; (2) 10-per-cent-butter fat-90-per-cent-galactose, and (3) 30-per-cent-butter fat-70-per-cent-galactose.

With the exception of rats on the 10-per-cent-butter fat-90-per-cent-galactose ration (who survived for only 12.6 days) all animals on the above rations developed paralysis and cataract as previously described for the dextrose-galactose series. No significant differences were noted between the present series and that already reported nor between animals on the 30-per-cent-butter fat-70-per-cent-galactose diet and those on the dextrose-galactose ration. It may be pointed out that the latter two diets were similar insofar as approximately 50 per cent of the calories in both rations were present as galactose. In addition to the paralysis already described, it was observed that approximately one-third of the paralyzed rats exhibited a loss of sensation in the tail. Animals were seen chewing the end of their tail and were unresponsive to the application of pressure with forceps or similar instruments. Histological sections were prepared of the gastrocnemius muscle of paralyzed rats. Sections were stained with hematoxylin and eosin and Mallory stain. No abnormalities were observed in the muscle fiber sufficiently pronounced to account for the paralytic condition. The following survival times were obtained for rats on the above rations: 10-per-cent-butter fat-90-per-cent-galactose 12.6 days (range 9 to 14 days); 50-per-cent-dextrose-50-per-cent-galactose 34.1 days (range 27 to 36 days); and 30-per-cent-butter fat-70-per-cent-galactose 33.2 days (range 24 to 43 days). No evidence was obtained under the conditions of the present experiment that butter fat had any effect on the incidence or severity of the "galactose paralysis."⁴

Further experiments have been conducted with rats of the Long-Evans strain. The experimental animals were similar in age and weight to those described above; 36 female rats were employed: 8 rats on lactose alone; 4 rats on galactose; 8 rats on dextrose; 8 rats on a diet of 50-per-cent-dextrose and 50 per cent galactose; and 8 rats on 30 per cent butter fat and 70 per cent galactose. No significant differences in length of survival were observed between the present series and the corresponding groups in the Sprague-Dawley strain. Significant differences were observed however in the incidence and severity of paralytic symptoms. No abnormalities in gait were observed before the 23rd

⁴ Similar results were obtained in subsequent experiments when corn oil was substituted for the butter fat of the above rations.

day of feeding; and severe paralysis was not observed before the 35th day. With one exception none of the Long-Evans rats were as severely affected as those of the Sprague-Dawley strain, although the majority of rats on dextrose-galactose and butter fat-galactose diets showed some degree of muscular paralysis or irregular gait. Differences were also observed in the incidence of cataract. Five of the 16 Long-Evans rats on dextrose-galactose and butter fat-galactose diets were albinos; and these developed mature cataracts after an average of 24 days. Cataracts were not observed in the remainder of the series.

Food and water intake. Table 2 lists the average daily food and water intake of Sprague-Dawley rats on experimental rations during the first four weeks of feeding.⁵ Subsequent to the first week a considerable degree of uniformity was observed in the caloric consumption of animals on the dextrose, sucrose and butter fat diets, while animals on the dextrose-galactose and butter fat-galactose rations consumed approximately twice this amount. It has been demonstrated that galactose feeding is associated with a marked increase in water consumption in the rat. In the present series, for example, water consumption on the galactose diet increased from 22 cc. on the first day of feeding when 4.7 grams of galactose were eaten to 128 cc. on the 7th day when 19 grams were ingested, a direct relationship existing between the amount of galactose eaten and the water consumed. The increased water consumption may be explained on the basis that rats were unable to utilize the amounts of galactose ingested and that water was required for its excretion in the urine.⁶ The increased food and water consumption of animals on the dextrose-galactose and butter fat-galactose diets may be explained therefore on the grounds that galactose calories were not utilizable and that animals ate more food in an effort to satisfy caloric requirements from non-galactose sources, the increased water intake being necessary for the excretion of galactose.

Discussion. Data obtained with the "single food choice" method may be objected to on the ground that this condition is never encountered normally in nature and that such data do not lend themselves to ready interpretation. Nevertheless physiological interrelationships may frequently be demonstrated far more efficiently under these simplified conditions than when animals are fed a more complete diet. Thus Richter and Rice (8) have demonstrated the specific rôle of thiamine hydrochloride in the utilization of carbohydrate by the rat; and the present experiment has confirmed with the single food choice method the cataractogenic effects of galactose. The method may be employed as a tool useful in indicating possible interrelationships subject to confirmation by other experimental procedures.

The present experiments suggest that the inadequacy of lactose as a dietary carbohydrate for the rat is due to an inability on the part of the rat to hydrolyze this disaccharide in adequate amounts. This is indicated by the fact that

⁵ Data for the Long-Evans strain was similar to that obtained for Sprague-Dawley rats on similar rations.

⁶ Holt and Kajdi (5) have identified galactose in the urine of rats fed a diet of galactose, with blood sugar reaching values as high as 550 mgm. per cent.

survival of animals fed lactose did not differ significantly from that of animals fed no food at all; and furthermore by the observation that ingestion of lactose was followed by diarrhea and marked distention of the cecum. When the products of hydrolysis—dextrose and galactose—were fed in place of the lactose, no diarrhea or cecal distention was observed, length of survival being substantially the same as that observed on the dextrose, sucrose or butter fat series.

Results indicate that galactose was not utilized as a source of calories by the rat, survival of animals on this sugar not differing significantly from that of animals fed no food at all; although the average caloric consumption ranged from 18 calories on the first day of feeding to 96 calories on the day preceding death. In addition animals fed a diet of dextrose-galactose or butter fat-galactose increased their food consumption to the extent that sufficient calories were obtained from non-galactose sources to meet body requirements. Water consumption on all galactose-containing diets was directly correlated with the amount of galactose ingested, indicating that galactose was not being utilized but excreted in the urine.

An unexpected finding was the occurrence of paralysis on dextrose-galactose and butter fat-galactose rations. Inasmuch as paralysis was not observed on diets of dextrose or butter fat alone, galactose appears to be the responsible agent. A strain difference was observed in the incidence and severity of this condition, animals of the Sprague-Dawley strain being more sensitive to galactose feeding than those of the Long-Evans strain.

SUMMARY

Female rats were fed diets consisting solely of 1, lactose; 2, galactose; 3, dextrose; 4, sucrose, and 5, butter fat in addition to a control group fed water alone. Length of survival for the lactose, galactose and fasting series averaged 5 to 7 days in contrast to a survival time of 34 ± 4 days for animals on the dextrose, sucrose or butter fat diets.

Evidence is presented indicating that rats failed to survive on lactose alone due to an inability to hydrolyze this disaccharide in adequate amounts. When the products of hydrolysis were fed in place of lactose, survival time was equal to that observed for the dextrose, sucrose or butter fat series.

Under conditions of the present experiment galactose was not utilized as a source of calories by the rat. When diets were fed consisting of 50-per-cent-dextrose-and-50-per-cent-galactose or 30-per-cent-butter fat-and-70-per-cent-galactose, food was ingested in sufficient amounts to meet body requirements with non-galactose calories. The non-galactose calories consumed on above diets were similar in amount to levels consumed on dextrose, sucrose or butter fat diets.

A severe flaccid paralysis was observed in rats fed dextrose-galactose or butter fat-galactose rations. Such paralysis did not occur on diets of dextrose or butter fat alone. A strain difference was observed in the incidence and severity of this condition.

REFERENCES

- (1) MITCHELL, H. S. J. Nutrition **12**: 447, 1936.
- (2) ERSHOFF, B. H. AND H. J. DEUEL, JR. J. Nutrition **28**: 225, 1944.
- (3) RICHTER, C. P., L. E. HOLT AND B. BARELARE. This Journal **122**: 734, 1938.
- (4) RICHTER, C. P. This Journal **133**: 29, 1941.
- (5) HOLT, L. E., JR. AND C. N. KAJDI. Bull. Johns Hopkins Hosp. **74**: 121, 1944.
- (6) SCHANTZ, E. J. AND C. F. KREWSON. Proc. Soc. Exper. Biol. and Med. **42**: 577, 1939.
- (7) SCHANTZ, E. J., C. A. ELVEHJEM AND E. B. HART. J. Biol. Chem. **122**: 381, 1938.
- (8) RICHTER, C. P. AND K. K. RICE. This Journal **143**: 336, 1945.

RELATION OF GAS TENSION AND HYDROSTATIC PRESSURE TO INTRAVASCULAR BUBBLE FORMATION¹

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The symptoms of compressed air illness, usually referred to as the "bends," have long been attributed to the occurrence of gas bubbles in the body. Paul Bert's (3) early contributions demonstrated that bubbles readily formed in the vascular system of animals when decompressed from high pressures. Later Haldane and his associates (4, 6), as well as Behnke and his co-workers (1), extended these investigations in an attempt to reduce the danger encountered by caisson workers and divers. More recently the same problem has been encountered when men have ascended to high altitudes.

Early investigations led Haldane to conclude that if "the excess of atmospheric pressure does not exceed about $1\frac{1}{2}$ atmospheres there is complete immunity from symptoms due to bubbles," regardless of the duration of exposure to the compressed air and the rapidity of decompression. He further reasoned that it would be just as safe to diminish the pressure rapidly from 4 atmospheres to 2, or 6 atmospheres to 3, as from 2 to 1. Later Boycott, Damant and Haldane (4) made tests first on goats and then on men and found that in general the results agreed with the hypothesis.

The present report is concerned with bubble formation in cats subjected to excess air pressures and then decompressed and observed at various lower pressures referred to as the hydrostatic pressure on the tissue. Its primary purpose is to determine under what conditions bubbles form with equal readiness over various compression-decompression ranges. An effort has also been made to correlate, as far as possible, bubble formation in cats with bends occurrence in man. The results do indicate that the pressure difference necessary for bubble formation in cats is the same as that for bends in goats and men.

The experiments are also instructive concerning the mechanism of bubble formation. Earlier theoretical considerations of bubble formation in isolated liquid systems at rest, as well as in animals, led to the conclusion that intravascular bubbles originated from gas nuclei. Further investigations indicated that the blood vessel walls were the important sites for these nuclei (9). The present results favor the view that in resting animals the gas nuclei from which bubbles come are not pre-existing gas phases but form at a relatively low pressure differ-

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ence (ΔP).⁴ The evidence for this conclusion will be brought out in the discussion.

MATERIALS AND METHODS. The general procedure has been to determine the incidence of bubble appearance in the blood of the exposed postcaval veins of nembutal anesthetized cats (0.045 gm. per kgm. intraperitoneally) after subjecting the animals to various experimental operations. The general techniques used in these studies have been described earlier where details are given (10). A new chamber has been used in these recent experiments which allows observations while the animals are under increased pressure as well as at simulated altitudes. The chamber was connected to both a vacuum and pressure reservoir so as to allow rapid pressure changes. As a measure of the degree of fatness of an animal the subcutaneous fat below the diaphragm plus the fat of the popliteal regions (designated as "subcutaneous" fat) has been removed and weighed and the results expressed as per cent of the body weight. The details of the fat analysis have been reported by Harvey et al. (10).

EXPERIMENTAL. *Pressurized cats decompressed to one atmosphere.* In this series 18 cats have been subjected to 3.5 atmosphere absolute of air pressure for 2 hours and then decompressed to one atmosphere. When ΔP is increased by increasing the gas tension alone (as with a resting cat), it is found that a pressure difference of 2.5 is necessary for bubble formation when the animals are observed at 1 atmosphere. The results for this series, recorded in table 1, indicate the relationship between fat and time of bubble formation. The fatter the cat the more rapidly do bubbles appear. It is probable, therefore, that many of the recorded negative cases would have been positive had the time of observation been extended.

Pressurized cats decompressed to 45,000 feet (0.14 atmosphere). Previous studies (10) have indicated that intravascular bubbles are rarely observed in resting cats which have been decompressed from ground level (1 atmosphere) to 45,000 feet (0.14 atmosphere), a ΔP of 0.86 atmosphere. However, bubbles appear quickly when the animals are exercised after this decompression. Since it was found necessary to increase ΔP by muscular contraction⁴ in order to obtain intravascular bubbles, it was of interest to determine what increase in ΔP (by increasing gas tension) would be necessary to obtain bubbles in a resting cat at this same final pressure. Two series of experiments at two different ΔP 's have been performed. The results are recorded in table 2. The cats were subjected to the high pressure (2.64 and 3.14 atmospheres) and then decompressed in a period of

⁴ The driving force for the separation of a gas phase, ΔP , has been defined as the difference between the dissolved gas tension, t , and the hydrostatic pressure, P , i.e., $\Delta P = t - P$ (8). The pressure may be either positive or negative and is measured from zero. In a resting animal subjected to 4 atmospheres gauge pressure (5 atmospheres absolute) of air and then decompressed to one atmosphere absolute ΔP is equal to $(5 - 1)4$. If the animal is stimulated, the contraction of muscles develops mechanical tensions, which may greatly lower P , and also forms excess CO_2 , which increases t . Both factors raise ΔP momentarily an unknown amount. It is, therefore, important to work with resting animals in which the spontaneous skeletal muscle contraction has been reduced. To meet these requirements we have worked with animals under anesthesia.

38 seconds to 45,000 feet (0.14 atmosphere) where the exposed postcavae were observed for the appearance of bubbles. The ΔP in the two series was 2.5 and 3.0, respectively. Oxygen was administered at the altitude by allowing a constant flow to pass over the tracheal cannula. The necessity of administering oxygen at 45,000 feet made these cats slightly different from those observed at 1 atmosphere, for in the latter the nitrogen gas tension in the lung is nearly equal to that in the ambient air, whereas in the cats at 45,000 feet the nitrogen tension in the lungs is reduced to nearly zero by the oxygen breathing after 15 to 20 minutes. This would cause the arterial blood of the cats breathing oxygen to be-

TABLE 1

Pressure-treated cats observed at one atmosphere

Time of bubble appearance in the postcavae of resting cats at one atmosphere after previous exposure to 3.5 atmospheres (absolute) for 2 hours. "Subcutaneous" fat is expressed as per cent total body weight. + indicates bubbles and - indicates no bubbles.

WT.	SEX	SUBCUT. FAT	TIME OF BUBBLES AFTER DECOMPRESSION
<i>kgm.</i>		<i>per cent</i>	<i>seconds</i>
2.72	♂	4.3	+1,260
3.41	♂	4.1	+595
2.90	♀	3.1	+300
2.66	♂	2.6	+980
2.65	♀	2.3	+1,600
1.76	♂	2.1	+2,340
1.44	♂	1.9	+2,690
2.35	♀	1.2	+3,540
2.50	♂	1.2	+3,780
1.57	♂	1.2	-3,000
2.86	♂	0.8	-3,000
2.79	♀	0.7	+5,220
2.32	♀	0.6	-3,000
2.97	♂	0.6	+5,400
2.88	♂	0.6	-3,180
2.70	♂	0.5	-3,240
2.74	♂	0.4	-5,000
1.92	♀	0.2	-3,000

come a more efficient medium for removing dissolved nitrogen from the tissue than would have been the case had air been breathed at 45,000 feet. Over a period of several minutes, however ΔP will be essentially the same in both cases. If bubbles are greatly delayed in formation there would be expected a slight difference between the two experiments.

It is interesting to observe that in those cases where bubbles did form they were not as delayed as in the experiments at 1 atmosphere. Also, in the altitude experiments, once bubbles appeared, it was a very short time (2-3 min.) until the postcava was completely filled with gas, whereas in the cats at 1 atmosphere usually the cavae did not completely fill with gas. At one atmosphere pre-

sumably the long delay in bubble appearance was related to their slow growth, whereas at the high altitude, once a gas phase had formed it grew rapidly and was soon visible in the postcava. In this connection it is also important to note the lack of a correlation between fat and time of bubble appearance in the animals decompressed to 45,000 feet.

Pressurized animals decompressed to 2 and 3 atmospheres. Cats have been observed at pressures above one atmosphere but with ΔP values equal to or greater than those used in the experiments where observations were made at 45,000 feet or 1 atmosphere. In the preliminary series of experiments, cats were

TABLE 2

Pressure-treated cats observed at 45,000 feet

Cats, with exposed postcavae, were compressed for two hours at high air pressures and then decompressed to 45,000 feet. Decompression from the high pressure was begun at zero time. At 30 seconds decompression to 45,000 feet was begun and the altitude was attained by 38 seconds. Time for bubble appearance is given from the time after reaching the simulated altitude, i.e., 38 seconds from zero time. + indicates bubbles and - indicates no bubbles. Oxygen was administered beginning at the time of decompression to 45,000 feet. "Subcutaneous" fat is expressed as per cent of body weight. In series A the cats were compressed at 2.64 atmospheres absolute and in series B they were compressed at 3.14 atmospheres absolute, which, upon decompression to 45,000 feet (0.14 atmosphere), gives a pressure difference of 2.5 and 3.0 respectively.

A. $\Delta P = 2.5$ ATMOSPHERES				B. $\Delta P = 3.0$ ATMOSPHERES			
Wt.	Sex	Subcut. Fat	Time of bubbles	Wt.	Sex	Subcut. Fat	Time of bubbles
kgm.		per cent	sec.	kgm.		per cent	sec.
2.60	♀	3.2	-4,000	2.38	♂	4.0	-4,000
2.90	♀	2.6	+152	2.24	♀	2.2	+307
2.44	♀	1.2	+262	3.36	♂	1.7	+322
2.35	♂	0.7	-4,000	2.62	♀	1.6	+1,582
2.23	♀	0.7	-4,000	2.41	♀	1.1	-4,000
2.20	♀	0.7	+202	1.93	♀	0.9	-4,000
2.04	♀	0.5	-4,000	3.24	♂	0.7	+192
2.50	♀	0.3	+747	2.20	♀	0.7	+262
1.88	♂	0.3	-4,000	1.85	♀	0.6	+1,122
1.92	♀	0.1	-4,000	2.39	♀	0.3	+702

subjected to air pressures ranging from 6 to 8 atmospheres absolute and were subsequently decompressed to 3 atmospheres absolute. This gave a range of ΔP from 3 to 5. Since bubbles did not appear in the blood vessels of these cats, the final pressure upon decompression was dropped to 2 atmospheres ($\Delta P = 5$ to 6). Still no bubbles were seen in the postcavae of these cats. The detailed results of these experiments are recorded in table 3. The time of observation after decompression was usually 1 hour, but in some cases the cats were observed for a longer period.

In an effort to obtain a ΔP that would be sufficient for bubble formation in cats resting under a pressure greater than one atmosphere a second series of experiments was performed. The air pressure applied was nine atmospheres absolute,

which was the maximum obtainable with our system, and was subsequently reduced to two atmospheres, giving a ΔP of 7.0. The results are recorded in table

TABLE 3

Pressure-treated cats observed at 2 or 3 atmospheres ($\Delta P < 7$)

Cats, with exposed postcavae, were compressed for two hours or longer at various high air pressures and then decompressed to 2 or 3 atmospheres where the exposed vessels were observed for the appearance of bubbles. + indicates bubbles and - indicates no bubbles. All times and pressures are recorded in the appropriate columns in the table. ΔP is the difference between the compression pressure and the decompression pressure, i.e., the gas tension minus the hydrostatic pressure. Time of bubbles is recorded with zero time beginning at the time of reaching the decompression pressure. The time necessary for decompression was 1 minute and 30 seconds. At the end of the observation time recorded in last column the cats were decompressed in 5 seconds to 1 atmosphere. "Subcutaneous" fat expressed as per cent of total body weight.

WT.	SEX	SUBCUT. FAT	DURATION OF COMP.	COMP. PRESSURE	DECOMP. PRESSURE	ΔP	TIME OF BUBBLES
kgm.		per cent	hrs.	atm. abs.	atm. abs.	atms.	sec.
2.62	♀	2.6	2.0	6	3	3	-3,600
2.55	♀	1.1	2.5	6	3	3	-3,600*
1.81	♂	2.4	3.0	7	3	4	-3,600
3.74	♂	2.1	2.0	7	3	4	-3,600*
1.67	♀	0.2	3.0	8	3	5	-3,600
2.01	♀	1.8	2.75	7	2	5	-5,400*
1.68	♀	0.5	2.5	7	2	5	-9,600
1.67	♀	1.4	3.0	8	2	6	-5,400

* Bubbles in postcava after 1 hour at 1 atmosphere.

TABLE 4

Pressure-treated cats observed at 2 atmospheres ($\Delta P = 7$)

Cats, with exposed postcavae, were compressed for at least two hours at 9 atmospheres absolute and then decompressed in 1 minute and 30 seconds to 2 atmospheres absolute where the exposed vessels were observed for the appearance of bubbles. Time of bubble appearance recorded from time of decompression. + indicates bubbles and - indicates no bubbles. "Subcutaneous" fat expressed as per cent of total body weight.

WT.	SEX	SUBCUT. FAT	DURATION OF COMPRESSION	TIME OF BUBBLES
kgm.		per cent	hrs.	sec.
2.51	♀	5.7	2.75	+490
2.18	♀	3.7	2.50	+1,190
2.07	♂	3.0	2.50	+1,080
2.42	♀	3.0	2.00	-5,400
3.08	♀	2.3	2.75	-5,400
2.35	♀	1.7	2.75	+1,680
2.96	♂	1.3	2.10	-7,200
2.63	♀	1.0	3.00	-5,400
2.93	♀	0.7	2.75	-4,200
2.05	♀	0.4	2.50	-3,600

4. As far as bubbles are concerned, they are comparable to those obtained at 1 atmosphere with a ΔP of 2.5, i.e., approximately 50 per cent positive. It is in-

teresting, however, that a ΔP of almost 3 times that at one atmosphere is necessary for bubble formation.

DISCUSSION. Boycott, Damant and Haldane found that compression to 6 atmospheres with subsequent sudden decompression to 2.6 atmospheres did not produce the symptoms of bends in goats. In a second series of experiments, where the drop of pressure was from 4.4 atmospheres to 1 atmosphere, only 20 per cent of their animals escaped symptoms. In comparing these results to the ones reported above, it is evident that the symptoms of bends in goats are directly paralleled by bubble appearance in cats. Thus, in the first series of Boycott, Damant and Haldane a ΔP of 3.4 did not produce symptoms when the observations were made at 2.6 atmospheres. Likewise, from table 3 above we see that bubbles do not form at ΔP 's ranging from 3 to 6 when the observations are made at 2 or 3 atmospheres. In Boycott, Damant and Haldane's second series, with a ΔP of 3.4 but observations at 1 atmosphere, 80 per cent of the goats showed symptoms. In cats observed at one atmosphere, a ΔP of 2.5-3 is necessary for bubble formation, i.e., the lower the hydrostatic pressure after decompression, the more susceptible are animals to decompression sickness. Haldane likewise states that it is evident that the whole danger lay in the last stages of the decompression. From the standpoint of bubble formation in cats it is apparent that the drop in pressure can be much greater than that used in the stage of decompression of Haldane when the pressures themselves are high. Decompression from 3 atmospheres to 1 atmosphere does not cause bubble formation, nor does decompression from 8 atmospheres to 2 atmospheres. If this same relationship holds over a wide range it would seem that decompression from 14 atmospheres to 3 atmospheres would fail to elicit bubbles. However, before subsequent decompression to 1 atmosphere it would be necessary to remain at 3 atmospheres for some time to allow for complete denitrogenation. In the experiments of table 3 where compression from 6 to 8 atmospheres was employed for 2 hours, it was necessary to remain at the lower pressure (2-3 atmospheres) for a longer period than an hour to avoid bubbles upon subsequent decompression to 1 atmosphere. In one of these experiments the cat was observed for $1\frac{1}{2}$ hours at 2 atmospheres after a 2-hour compression at 7 atmospheres without bubbles appearing. The cat was then decompressed to 1 atmosphere, and after one hour bubbles appeared in the postcava. Usually no bubbles appear when the pressure change is from 2 to 1 atmosphere. The results of other experiments similar to these emphasize that it is, as Haldane stated, the final stages of decompression that are dangerous.

Since the problem of preventing bubble formation in compression-decompression experiments is one of elimination of dissolved gases, Haldane and his associates suggested muscular exercise during decompression in order to increase the rate of circulation and consequently the rate of desaturation. However, since recent studies have demonstrated that muscular activity facilitates bubble formation in various animals that have been decompressed to simulated altitudes (10, 13), it is important to investigate the problem of exercise in compression experiments.

Harvey et al. (10) have previously demonstrated that muscular contraction is

more effective in initiating intravascular bubble formation under conditions of reduced pressure, such as high altitudes, than at 1 atmosphere pressure. Bubbles rarely occurred in cats resting for 1 hour at 45,000 to 50,000 feet, whereas they appeared rapidly when the animals were exercised at these altitudes. In contrast, in resting cats decompressed from 3.5 atmospheres to 1 atmosphere, bubbles were apparent in about 50 per cent of the animals in less than an hour, and exercise, while accelerating bubble appearance under these conditions, did not increase their incidence. Further, in cats decompressed from 3 atmospheres to 1, even exercise failed to elicit bubble formation (11). It is evident, then, that exercise, with the strength of contraction used in our experiments, does not greatly facilitate bubble formation at one atmosphere, i.e., it does not cause bubbles to form where they would not occur without it. It may be concluded that, as the hydrostatic pressure after decompression becomes increasingly higher, the less effective will be muscular activity in facilitating bubble formation. This is to be expected if contraction is favoring bubble formation by further reducing the hydrostatic pressure; for, in accordance with Boyle's law, any additional reduction of pressure on gas cavities present in cats already under partial vacuum will increase their volumes proportionately more than would the same pressure reduction at 1 atmosphere pressure.

There is little doubt that vigorous muscular activity greatly facilitates removal of dissolved nitrogen from most parts of the body, although there is evidence that denitrogenation of fat tissue is not facilitated and in some cases may be slowed by this activity (15). However, it seems likely, as Haldane states, that exercise will have a beneficial effect during decompression from high pressures and that this beneficial effect will more than compensate for the possible deleterious effects discussed above. Theoretically, however, the more vigorous exercise should be employed at the higher pressures and only light exercise be used as one approaches 1 atmosphere, for it is possible to obtain bubbles at the latter pressure even without previous high gas pressure treatment. This has been demonstrated to be the case with crushed legs or broken bones (11). Berg et al. (2) have confirmed the finding that leg crushing and bone breaking resulted in the formation of gas bubbles. With a greater lowering of the hydrostatic pressure than provided by the type of exercise used in the experiments with cats there is no doubt that exercise would greatly facilitate bubble formation even under conditions of low dissolved gas tension, a view supported by other experiments (6, 7, 12, 13, 14). Again, however, this is merely a restatement of the relationship of gas tension to hydrostatic pressure necessary for bubble formation.⁴

The results of the experiments reported in this paper clarify considerably the question of whether intravascular bubbles arise from pre-existing gas nuclei or form *de novo* upon decompression. The similarity of results between the experiments detailed in table 1 (cats decompressed from 3.5 atmospheres and observed at 1 atmosphere) and table 2, A (cats decompressed from 2.64 atmospheres and observed at 0.14 atmosphere) is especially significant. If pre-existing gas nuclei had been present in these cats, clinging to hydrophobic surfaces or lodged in crevices, then the initial compression to 3.5 and 2.64 atmospheres would have

immediately compressed them, increasing their internal gas pressures. Because of this, and the fact that the increased gas pressure in the lungs would not result in an equivalent gas tension in the blood at the sites of the nuclei for some minutes, there would be a net diffusion of gas out of the nuclei into the blood, and the critical pressure difference, ΔP_c , for the nuclei upon subsequent decompression would then be larger.⁵ If the liquid around these nuclei desaturates too rapidly after decompression the critical pressure difference may not be reached and the nuclei therefore may not be able to grow and give rise to visible bubbles. In the two experiments under consideration, however, this factor is of quite different magnitudes. Although a ΔP of 2.5 is obtained in both experiments, in the first any pre-existing nuclei were exposed to $3.5 - 2.64 = 0.86$ atmosphere more pressure than in the second. Further, in the second experiment, nuclei would be expanded approximately 7 times more upon decompression than would those in the first, according to Boyle's law. Both of these factors would tend to facilitate bubble formation in the second experiment, where observations were made at a simulated high altitude, if we were dealing with preformed gas nuclei. The results show definitely that this is not the case; bubbles form with equal readiness in both experiments. It is understandable why similar results are obtained in the two experiments if we are concerned with formation of gas nuclei after decompression, rather than preformed ones.

Therefore it appears probable that in nembutal anaesthetized animals (i.e., resting animals) intravascular bubbles arise from new gas nuclei, rather than from pre-existing ones, and it is only under conditions of activity at relatively low pressures (1 atmosphere or less) that pre-existing nuclei may persist. Exercise prior to ascent to high altitude is recognized as a pre-disposing factor to bends. The degree of exercise and rest prior to ascent is undoubtedly one of the many factors which give rise to the variability of bends and bubble formation observed in men and experimental animals.

SUMMARY

The effect of hydrostatic pressure (i.e., the pressure of final decompression) on the formation of intravascular gas bubbles has been studied in animals after various degrees of gas (air) saturation. When resting animals are observed at one atmosphere a pressure difference (gas tension minus hydrostatic pressure) of 2.5 is necessary for bubble formation. When the hydrostatic pressure is reduced to 111 mm. Hg (45,000 ft.) a pressure difference of 2.5 is again necessary for spontaneous bubble formation in the vascular system of resting animals. When resting animals are observed at higher pressures it is found that a larger pressure difference is necessary for bubble formation. When observations are made at 2 atmospheres a pressure difference of 7 is limiting, i.e., the animals are

⁵ The critical pressure difference (ΔP_c) is the pressure difference (dissolved gas tension minus hydrostatic pressure) necessary to render a given nucleus unstable so that it will grow indefinitely and thus form a bubble. It will depend on the configuration and hydrophobicity of the surface to which the nucleus is attached and on the surface tension.

compressed with an air pressure of 9 atmospheres absolute and subsequently decompressed to 2 atmospheres absolute.

It is demonstrated that bubble formation occurs under conditions similar to those in which "bends" occur. The results are discussed in relation to the problem of decompression sickness, as well as to the mechanism of bubble formation. The evidence favors the view that intravascular bubbles originate from gas nuclei which form as a result of the gas supersaturation, rather than from pre-formed gas masses.

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REFERENCES

- (1) BEHNKE, A. R. Harvey Lectures **37**: 198, 1942.
- (2) BERG, W. E., M. HARRIS, D. M. WHITAKER AND V. C. TWITTY. J. Gen. Physiol. **28**: 253, 1945.
- (3) BERT, P. La Presson Barometrique. Paris, G. Masson, 1878.
- (4) BOYCOTT, A. E., G. C. C. DAMANT AND J. S. HALDANE. J. Hygiene **8**: 342, 1908.
- (5) HALDANE, J. S. AND J. G. PRIESTLEY. Respiration. Yale University Press, New Haven, 1935.
- (6) HARRIS, M., W. E. BERG, D. M. WHITAKER, V. C. TWITTY AND L. R. BLINKS. J. Gen. Physiol. **28**: 225, 1945.
- (7) HARRIS, M., W. E. BERG, D. M. WHITAKER AND V. C. TWITTY. Ibid. **28**: 241, 1945.
- (8) HARVEY, E. N., D. K. BARNES, W. D. McELROY, A. H. WHITELEY, D. C. PEASE AND K. W. COOPER. J. Cell. and Comp. Physiol. I, **24**: 1, 1944.
- (9) HARVEY, E. N., A. H. WHITELEY, W. D. McELROY, D. C. PEASE AND D. K. BARNES, Ibid. II, **24**: 23, 1944.
- (10) HARVEY, E. N., W. D. McELROY, A. H. WHITELEY, G. H. WARREN AND D. C. PEASE. Ibid. III, **24**: 117, 1944.
- (11) McELROY, W. D., A. H. WHITELEY, G. H. WARREN AND E. N. HARVEY. Ibid. IV, **24**: 133, 1944.
- (12) McELROY, W. D., A. H. WHITELEY, K. W. COOPER, D. C. PEASE, G. H. WARREN AND E. N. HARVEY. Ibid. VI, **24**: 273, 1944.
- (13) WHITAKER, D. M., L. R. BLINKS, W. E. BERG, V. C. TWITTY AND M. HARRIS. J. Gen. Physiol. **28**: 213, 1945.
- (14) WHITELEY, A. H., W. D. McELROY, G. H. WARREN AND E. N. HARVEY. J. Cell. Comp. Physiol. V, **24**: 257, 1944.
- (15) WHITELEY, A. H. AND W. D. McELROY. This Journal, in press.

THE OXYGEN CONSUMPTION AND MECHANICAL EFFICIENCY OF THE HEART BEFORE AND DURING HEART FAILURE¹

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I. MECHANICAL EFFICIENCY AS THE HEART FAILS. Knowlton and Starling (1) showed that the work of the heart is proportional to the diastolic volume. This has been confirmed by Wiggers and Katz (2). Expressed as the Starling "Law of the Heart": the mechanical energy set free at each contraction of the heart is a simple function of the diastolic volume of the heart. This was found to be true up to an optimum diastolic volume beyond which the work decreased. In the frog heart, Anrep and Segall (3) showed that the tension developed in isometric contraction was proportional to the initial (diastolic) tension up to a certain point. Katz (4) in the turtle heart was able to distinguish between initial fibre length and fibre tension and demonstrated that initial length was the factor determining the force of contraction.

Starling and Visscher (5) contended that the oxygen consumption of the heart muscle, that is, the total energy input, is directly proportional to the diastolic volume and that the ratio—O₂ consumption/diastolic volume—remains constant though diastolic volume varies widely, whether or not the heart is in failure. Their observations forced them to the conclusion that, as the heart goes into failure and the work decreases while the diastolic volume increases, there is a decrease in mechanical efficiency due to both a progressively smaller numerator and to a progressively larger denominator in the equation:

$$\text{mechanical efficiency} = \frac{\text{work}}{\text{O}_2 \text{ consumption.}}$$
 The conclusions reached by Peters

and Visscher (6) and by Fahr and Buehler (7) in support of this assertion may be questioned because they measured only that part of the cardiac output which passes into the aorta beyond the mouths of the coronary arteries. The occurrence of spontaneous coronary dilatation in isolated heart and heart-lung preparations has been noted and such coronary dilatation has been found by us to be common in the course of an experiment (8) even while failure is occurring (9), so that the work of the heart calculated from aortic flow may appear to be reduced and the mechanical efficiency appear to be decreased because the work of the heart is underestimated. The sources of error in the measurement of O₂ consumption by the indirect method of Starling and Visscher have been enumerated previously (10).

In this laboratory, using isolated heart and heart-lung preparations, we have

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not been able to confirm the findings of Starling and Visscher. In the isolated heart Katz and Mendlowitz (10) permitted heart failure to occur under conditions 1, where there was little or no change in diastolic volume, and 2, where the diastolic volume increased but the work of the heart was kept constant, and found in the former case a progressive decrease in work and oxygen consumption, and in the latter case no change in oxygen consumption; in both types of experiment there was little or no change in mechanical efficiency during failure. Katz, Jochim, Lindner and Landowne (11) showed in both the isolated heart and heart-lung preparations that the effect of altering the work on the oxygen consumption and mechanical efficiency in the absence of heart failure depended on whether the work was changed by varying either the venous return—i.e., cardiac output, or the peripheral resistance—i.e., the arterial pressure. When cardiac output was increased, the rise in O_2 consumption was less than the rise in work so that the mechanical efficiency tended to increase. On the other hand, when arterial pressure was raised, the rise in O_2 consumption was approximately proportional to the rise in work so that the mechanical efficiency showed little or no change.

The errors inherent in measuring work and oxygen consumption in perfused hearts have been discussed at length in previous communications from this laboratory (10, 11, 12, 13) and will not be reiterated here. The objection has been raised to our early studies on heart failure, that the coronary sinus sample used by us as an index of venous oxygen was not a true mixed venous sample⁴ (14). Therefore, in subsequent work (11) oxygen consumption was calculated from the product of pulmonary flow and the oxygen difference between systemic arterial blood and venous blood from the pulmonary artery. Using this method, we were able to substantiate our previous results, namely, that at constant work levels, the failing heart even though it increases in size, does not show any appreciable change in oxygen consumption and therefore its mechanical efficiency is not altered significantly (15).

This report will deal with further data on oxygen consumption and mechanical efficiency. The method of establishing these preparations and making the measurements has recently been described in some detail (8, 9, 16 and 17). The method of calculating work, O_2 consumption and mechanical efficiency has been dealt with elsewhere (10 and 11).

Obviously, it was necessary to keep the work of the heart constant while it went into failure, in order to eliminate from the comparison those changes in mechanical efficiency which accompany changes in the work performed. In the heart-lung preparation this was accomplished by adjusting the amount of blood in circulation, and in the isolated heart preparation by adjusting the screw clamp between the inflow reservoir and the preparation. In the isolated heart preparation, the work level could actually be adjusted to within a few percent. In the heart-lung preparation, satisfactory constancy was achieved in all but two preparations in which work rose during the failure period (cf. table 1B).

⁴ This objection does not apply to our studies on the effect of changing the work of the non-failing heart (11).

In another report (18) a theoretical evaluation of O_2 consumption as an index of energy input and the significance of mechanical efficiency has been made. Certain assumptions are made in calculating energy and efficiency. Recognizing that these assumptions may be invalid, it is still important to decide whether or not a change in energy input and mechanical efficiency so calculated is present in failure for a given heart size and work load.

Results of present investigation. In the present report data have been assembled on all experiments in which the heart rate was stable and data were

TABLE 1A

Work, O_2 used and efficiency of control and failure periods compared—isolated heart preparation

NO. OF EXPERIMENT	WORK (KGM/HR.)				OXYGEN CONSUMPTION (KGM/HR.)				EFFICIENCY (%)			
	Control	Failure	Average		Control	Failure	Average		Control	Failure	Average	
			Control	Failure			Control	Failure			Control	Failure
43	15.53	15.38 14.93	15.53	15.16	480	439 356	480	398	3.24	3.50 4.19	3.24	3.85
44	16.10 15.88	16.44 16.57 15.96	15.99	16.32	486 443	376 422 399	465	399	3.31 3.59	4.38 3.93 4.01	3.45	4.11
47	16.62	17.20 16.81	16.62	17.01	300	403 463	300	433	5.56	4.27 3.64	5.56	3.96
49	13.99	14.24	13.99	14.24	511	527	511	527	2.74	2.70	2.74	2.70
50	16.23 15.79	16.50 17.04	16.01	16.77	396 339	294 257	368	276	4.10 4.66	5.61 6.66	4.38	6.14
51A	8.33	6.47	8.33	6.47	531	399	531	399	1.57	1.62	1.57	1.62
51	9.77 9.77 12.02 12.21	9.95	10.94	9.95	319 365 371 343	403	350	403	3.06 2.68 3.24 3.58	2.47	3.14	2.47
53	20.41	20.55 20.04 18.51 18.42	20.41	19.38	387	333 451 399 207	387	348	5.27	6.17 4.44 4.63 8.90	5.27	6.04
Averages...			14.73	14.41			424	398			3.67	3.86

available in the control and failure periods. A few experiments previously reported (11 and 15) were included. The values of these experiments were divided into four groups: *a*, those obtained during the control period in the isolated heart, and *b*, during this period in the closed heart-lung preparation: *c*, those obtained during heart failure in the isolated heart, and *d*, during this period in the closed heart-lung preparation.

A total of eight isolated heart experiments and eleven closed heart-lung experiments were used. The values of each period were averaged in each experiment. In the heart-lung preparation the 11 values for the control periods

were based on 1 to 3 determinations (average 1.6) and those for the failure periods were based on 1 to 6 determinations (average 2.2). In the isolated heart preparation the 8 values for the control periods were based on 1 to 4 determination (average 1.6) and those for the failure periods on 1 to 4 determinations (average 2.0). The pertinent data are shown in table 1A and B.

TABLE 1B

Work, O₂ used and efficiency of control and failure periods compared—heart-lung preparation

NO. OF EXPERIMENT	WORK (KGM/HR.)				OXYGEN CONSUMPTION (KGM/HR.)				EFFICIENCY (%)			
	Control	Failure	Average		Control	Failure	Average		Control	Failure	Average	
			Control	Failure			Control	Failure			Control	Failure
55	10.39	24.62 27.75	10.39	26.18	611	1229 532	611	881	1.70	2.00 5.22	1.70	3.61
61	9.84 10.32	12.00	10.08	12.00	220 288	657	254	657	4.47 3.58	1.83	4.03	1.83
62	16.52	15.68 19.37	16.52	17.53	737	1115 1092	737	1104	2.24	1.41 1.77	2.24	1.59
64	32.80 32.80	37.91 40.23	32.80	39.07	864 768	1048 936	816	992	3.80 4.27	3.62 4.30	4.04	3.96
66	13.85 14.69	16.82 18.88	14.27	17.85	265 447	231 380	356	306	5.23 3.29	7.29 4.97	4.26	6.13
68	14.49	14.52 15.42	14.49	14.97	566	447 356	566	402	2.56	3.25 4.33	2.56	3.79
23	7.89 6.41 7.82	7.26 7.54 7.71	7.37	7.49	626 434 368	365 509 425	476	431	1.26 1.48 2.13	1.99 1.48 1.81	1.62	1.76
		7.80 6.97 7.64				442 358 489				1.76 1.95 1.56		
24	16.27	20.32 20.96 21.25	16.27	20.84	354	567 544 458	354	523	4.60	3.59 3.85 4.64	4.60	4.03
25	46.26 42.87	44.94	44.57	44.94	518 683	563	601	563	8.93 6.29	7.98	7.61	7.98
26	29.88 34.93	45.76	32.41	45.76	677 667	635	672	645	4.41 5.24	7.20	4.83	7.20
31	37.03	22.11 20.39	37.03	21.25	1443	976 590	1443	783	2.57	2.26 3.46	2.57	2.86
Averages...			21.47	24.35			626	662			3.64	4.07

In the isolated heart preparation the average value for mechanical efficiency calculated from the figures so obtained was 3.67 for the control period and 3.86 for the period of failure. This difference is obviously insignificant. In 5 experiments the efficiency increased as failure developed, in 3 it decreased; thus, there is a random change in the efficiency when failure develops.

In the heart-lung preparation the average value for mechanical efficiency was

3.64 per cent for the control period and 4.07 per cent for the period of heart failure, again an insignificant difference. Here also there are random changes; in 7 experiments the efficiency increased as heart failure developed, in 4 it decreased.

Omitting from the calculation of the heart-lung preparations the two in which the work level rose significantly when the heart was in failure, the average figures of table 1 B read as follows:

Average work level in the control period	= 21.49 kgm/hr.
Average work level in the failure period	= 21.77 kgm/hr.
Average oxygen consumption in the control period	= 623 kgm/hr.
Average oxygen consumption in the failure period	= 640 kgm/hr.
Average mechanical efficiency in the control period	= 3.75 per cent
Average mechanical efficiency in the failure period	= 3.77 per cent

It is clear from the above figures that the maintenance of the mechanical efficiency as the heart goes into failure is not due to increased work levels. Therefore, when the work level is unchanged, the mechanical efficiency does not decline as the heart fails.

These data therefore support the contention previously expressed by us that a decline in mechanical efficiency is not the *constant* accompaniment of heart failure, as is implied by the work of Starling and Visscher (5) and their school.⁵

II. MECHANICAL EFFICIENCY AND THE WORK LEVEL. We have previously investigated the changes in the efficiency of the heart under conditions of changed work loads (11) and found that the efficiency improves with higher work loads if the increased work is due to increased venous return and therefore to increased cardiac output. On the other hand, the efficiency remained stationary or even declined if increased work was required in order to overcome an increased resistance load. The data collected in the present report for the purpose of comparing mechanical efficiency before and during heart failure offer the opportunity for further study of mechanical efficiency under varied work loads accompanying variation in the cardiac output.

The positive correlation between efficiency and work level has commonly been ascribed to the existence of a significant fixed resting oxygen consumption of heart muscle. As work is increased, the oxygen consumption of the preparation is assumed to go up in proportion to the work increase. The extra burden of the resting oxygen therefore constitutes a smaller and smaller fraction of the oxygen total, permitting the efficiency to increase. For the case of work increase due to increase in resistance load this would of course have to hold too, but it is conceivable that some other train of events is set up in contraction against

⁵ In forward failure (for definition see (9)), when the work of the heart and the cardiac output decrease, mechanical efficiency would decrease just as it does when cardiac output and work decline in a non-failing heart. Whether the mechanical efficiency drop under such circumstances would be greater, less or the same as for a similar decline in work and cardiac output in the non-failing heart we cannot say. All but one of the experiments analyzed in this report had only congestive failure and no forward failure during the time observations were made, since the attempt was made to keep the work and cardiac output constant. Observations to settle this matter definitely are necessary.

higher pressure which impairs the efficiency of contraction and thus cancels or even overbalances the gains made by the greater work load.

The present investigation cannot throw any light on this latter problem as the attempt was made to keep aortic pressure constant. Actually there was very little fluctuation in aortic and pulmonary arterial pressures in these experiments and the variations in work levels are mainly due to variations in cardiac output.

If the positive correlation between efficiency and work, where it is found, is considered to be due entirely to the diminished weight of the resting oxygen consumption in the oxygen total, that is, that the energy yield per unit oxygen used for work is thought to be the same for a given heart at any work level, then the efficiency-work curve is given by the equation:

$$E = \frac{W}{O_0 + O_w}$$

in which $\frac{W}{O_w}$ equals a constant C . E in this equation is the efficiency, W is the work, O_0 and O_w are respectively the O_2 consumption for the resting and work metabolism of the heart. Substituting for O_w its value $\frac{W}{C}$, this equation becomes:

$$E = \frac{WC}{O_0C + W}$$

This equation expresses curves in which E increases indefinitely as W increases, but whose slopes, while always positive, decrease steadily. Slope and level of the curves depend, of course, on the magnitudes of the constants O_0 and C . It can be seen that with the magnitudes selected for our sample curves (fig. 1) and for the work range under consideration, an increase in O_0 will make for a steeper slope of the curve while lowering its level. The corresponding curves of total oxygen against work, while always straight lines, will be of identical slope, with the highest level for the highest O_0 . Conversely, if the resting oxygen consumption O_0 remains constant, but the energy yield per unit oxygen used for work, C , varies from 0.1 to 0.0667, the slope of the efficiency-work curve remains practically the same, and the curve is lifted to higher levels for higher C . The corresponding oxygen curves, on the other hand, change slope with C and are steepest where C is lowest.

The assumptions underlying the above discussion appear to us reasonable enough to warrant examination of our data in terms of this hypothesis. As a first approximation to realistic values for the constants O_0 and C —we have used the average and, at the same time, the most frequent figures for work and oxygen consumption found in our preparation; an oxygen consumption of 400 kgm./hr. corresponding to a work level of 20 kgm./hr. If of the total oxygen of 400 units, 100 and 200 kgm. respectively are assigned to resting oxygen consumption, and C is selected such as to make the efficiency 5 per cent at the work level of 20 kgm./hr., two hypothetical curves are obtained in which for a work variation of from 8 to 46 kgm./hr. there is an efficiency variation of from 3.6

to 5.8 and from 2.8 to 7.0 per cent. Both curves are flattened out strongly at the highest work levels. The rather small variation in efficiency over the wide range of work levels should be noted (fig. 1). The corresponding O_2 curves are also shown in this figure.

Our data are not suited for the attempt to fit the figures obtained in single hearts to some such curve because of the narrow range of work levels in any single experiment and the comparatively large error contained in the oxygen and therefore also the efficiency data. As stated above, work was kept constant,

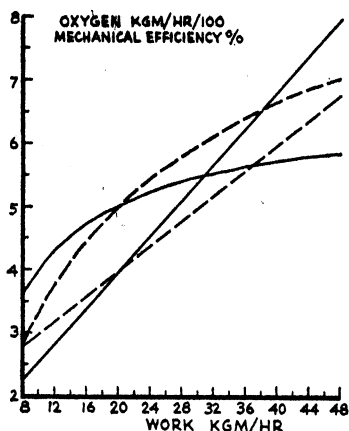


Fig. 1

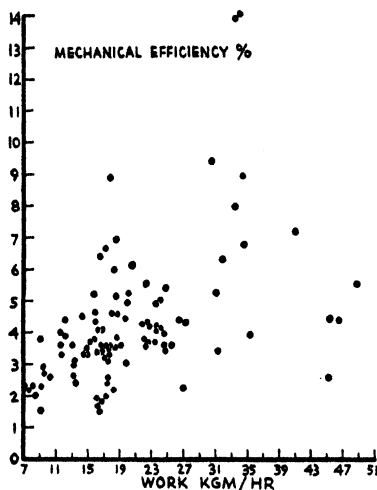


Fig. 2

Fig. 1. Two sets of hypothetical curves relating mechanical efficiency and oxygen consumption to work. The dash curves are constructed on the assumption that the resting oxygen consumption is 200 kgm/hr. and that the constant $C = 0.1$; the solid curves are constructed on the assumption that the resting oxygen consumption is 100 kgm/hr. and $C = 0.0667$. In each set of curves the curvilinear line represents the relation of efficiency to work and the straight line, the relation of oxygen consumption to work. Discussed in text.

Fig. 2. Scattergram of all data relating efficiency to work in both the isolated heart and heart-lung preparations. Discussed in text.

except where increase in cardiac output was necessary to maintain the aortic pressure.

In comparing different preparations, the $E-W$ curves will coincide if the two constants O_0 and C are identical for the hearts under comparison. Plotting all our efficiency values from all preparations against their associated work values should result in a single curve with some scatter, if all the hearts have the same resting oxygen values and the same work yield per cubic centimeter of oxygen. The oxygen values plotted against work should be a straight line of a slope predictable from the $E-W$ figures. Our actual data, when so plotted, form an asymptotic curve of extremely wide scatter, but show no correlation at all

between oxygen consumption and work (fig. 2). Our hearts can therefore not be considered to have the same constants O_o and C .

As to the resting oxygen consumption O_o , one would expect variation with the weight of the heart if not with other factors. As to the conversion factor C , one might assume that at least in the same species, i.e., in animals with the same metabolic processes, the energy yield per cubic centimeter of oxygen might be the same for all hearts. However, C does not merely express the energy yield of oxygen, but more specifically the work yield from a given energy supply. If large differences in mechanical efficiencies exist in intact animals of the same heart size, they must be due to differences in C which under the above assumptions is the main factor determining efficiency. In the isolated heart or heart-lung, it is obvious that there will be differences in the state of the preparation resulting from the variable insults suffered in the surgical procedure, the variable degree of compatibility of the perfusate, the variable temperature and moisture at which the preparation is kept, etc. One might expect all these factors, which show up in great differences in performance and also survival time, to express themselves as differences in both C and O_o , but particularly in the former.

When our actual E - W data are examined⁶, it is obvious that differences in the resting O_o alone can not explain the very wide scatter nor the very wide range over which E varies. Even for the steepest of our hypothetical curves, discussed above, the total efficiency range is slightly over 4 per cent with a work range of from 8 to 46 kgm.

A suggestion concerning the additional factor was obtained when the various preparations were arranged in increasing order of their average efficiency. When this is done, the corresponding W -values are found to increase up to a point, then drop to a low value and again increase, then drop to a low value, and again increase, then drop and increase again. The breaks suggest a corresponding grouping of the hearts. Within each of the three groups, efficiency varies over a sufficiently narrow range that one might expect the constants O_o and C to be sufficiently close to one another to fit a single curve.

In plotting the E - W values for each of the groups so obtained, the points arrange themselves with greatly diminished scatter. Lines can be drawn through the scatter with reasonable certainty. Their slopes, while different for each group, fit equations of the type discussed above (figs. 3, 4 and 5). The equations which these curves fit can be estimated from their slope which indicates the magnitude of O_o , the magnitude of C then being computed from any one point of the curve. From the equation relating E and W , the slope of the line relating total oxygen consumption and work was computed for each group. It is seen from the graphs that efficiency and especially oxygen data fit their predicted curves at least in slope and shape. More than this should not be expected, because the constants are presumably only very approximately alike

⁶ For this analysis a total of 12 isolated heart preparations and 13 closed heart-lung preparations were used. The data of the two types of preparations were combined as the ranges and trends were very similar in both.

for the different hearts assembled in each group. For this reason the grouping of the hearts is arbitrary to some degree, as is also the exact value assigned to the constants in each group.

The fact that approximate fits for oxygen and efficiency data were obtained from the assumptions made above tends in turn to support the assumptions, viz., that efficiency improves with increased work because of the existence of a significant resting oxygen consumption whose weight diminishes in the oxygen

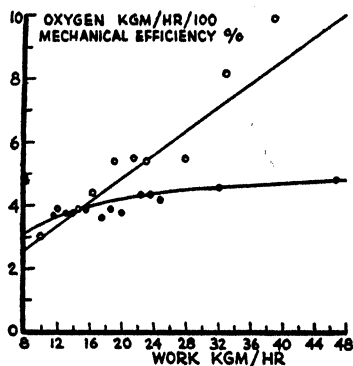


Fig. 3

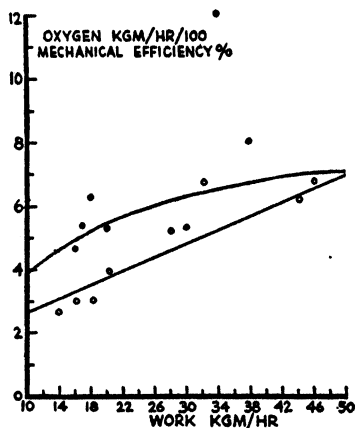


Fig. 4

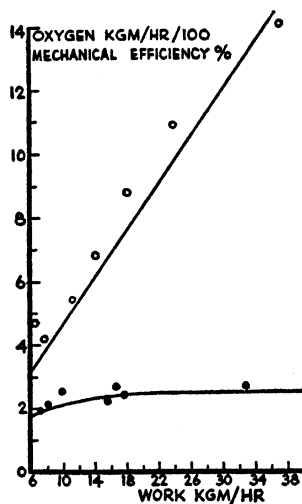


Fig. 5

Fig. 3. Comparison of theoretical curves with actual data in experiments having an average efficiency between 3 and 5 per cent. The assumptions in creating the curve relating O_2 (straight line) and efficiency (curvilinear line) to work are: resting $O_2 = 106$ kgm/hr. and $C = 0.0533$. The actual observations in these experiments are shown by the open circles and solid circles respectively, relating O_2 consumption and efficiency against work. Discussed in text.

Fig. 4. Comparison of theoretical curves with actual data in experiments having an average efficiency of 5 per cent or over. Constructed as in figure 3. The assumptions for the theoretical curves are: resting $O_2 = 150$ kgm/hr. and $C = 0.0906$. Discussed in text.

Fig. 5. Comparison of theoretical curves with actual data in experiments having an average efficiency of 3 per cent or less. Constructed as in figure 3. The assumptions for the theoretical curves are: resting $O_2 = 101$ kgm/hr. and $C = 0.0275$. Discussed in text.

total as the work and therefore the total oxygen increases. Our analysis however also shows that the main factor which makes the efficiency of one heart different from that of another heart is not the magnitude of the resting oxygen fraction. Thus the values of resting oxygen assumed in the three groups, viz. 101, 106 and 150 kgm/hr. respectively, constitute respectively, $\frac{1}{3}$, $\frac{1}{4}$ and $\frac{1}{5}$ of the average total oxygen used in the three groups. The conversion factor C , the work yield per cubic centimeter of oxygen used, on the other hand, is 0.0275 for the first group, 0.0533 for the second group and 0.0906 for the third group. Efficiency in

the lowest efficiency group therefore is low in spite of a low resting oxygen because of the poor work yield per unit of oxygen. Conversely in the highest efficiency group, efficiency is high in spite of a high resting oxygen consumption. A high resting oxygen rather seems to indicate that the metabolic processes in a preparation are not depressed and that the preparation is therefore capable of performing work efficiently.

This analysis therefore suggests that the scatter of efficiency of the different hearts is due as much to variation in the efficiency of conversion of oxygen to energy and energy to work as it is due to variation in the energy used to maintain the resting state of the heart.

SUMMARY

1. Further experiments were carried out on the isolated heart and the closed heart-lung preparations under conditions of constant work and constant aortic pressure in order to compare the mechanical efficiency of the failing heart with its efficiency before failure develops. Repeated efficiency determinations in 8 isolated heart and 11 heart-lung preparations fail to reveal any significant differences in efficiency between control and failure periods.

2. The effect upon mechanical efficiency of different work loads imposed upon different hearts was investigated in the same preparations. It was found that for hearts whose efficiency at a certain work level is similar, efficiency is an asymptotic function of work according to the equation: Efficiency equals $\frac{\text{work}}{\text{resting oxygen plus oxygen used for work}}$ if $\frac{\text{work}}{\text{oxygen used for work}}$ is considered to be constant for each heart. The hearts used in this study have been arranged into three efficiency groups. The values for resting oxygen consumption and for the constant $\frac{\text{work}}{\text{oxygen used for work}}$ have been estimated for each group, and efficiency and oxygen curves have been calculated according to the above equation. A fair degree of agreement between the calculated and the experimental curves was found.

REFERENCES

- (1) KNOWLTON, F. B. AND E. H. STARLING. *J. Physiol.* **44**: 206, 1912.
- (2) WIGGERS, C. J. AND L. N. KATZ. *This Journal* **58**: 439, 1922.
- (3) ANREP, G. AND H. N. SEGALL. *Heart* **13**: 61, 1925.
- (4) KATZ, L. N. *This Journal* **87**: 348, 1928.
- (5) STARLING, E. H. AND M. B. VISSCHER. *J. Physiol.* **62**: 243, 1926.
- (6) PETERS, H. C. AND M. B. VISSCHER. *Am. Heart J.* **11**: 273, 1936.
- (7) FAHR, G. AND M. S. BUEHLER. *Am. Heart J.* **25**: 211, 1943.
- (8) KATZ, L. N., W. WISE AND K. JOCHIM. *This Journal* **143**: 479, 1945.
- (9) KATZ, L. N., W. WISE AND K. JOCHIM. *This Journal* **143**: 507, 1945.
- (10) KATZ, L. N. AND M. MENDLOWITZ. *This Journal* **122**: 262, 1938.
- (11) KATZ, L. N., K. JOCHIM, E. LINDNER AND M. LANDOWNE. *This Journal* **134**: 636, 1941.
- (12) KATZ, L. N., M. MENDLOWITZ AND H. A. KAPLAN. *Am. Heart J.* **16**: 149, 1936.

- (13) KATZ, L. N., K. JOCHIM AND A. BOHNING. This Journal **122**: 236, 1938.
- (14) MOE, G. K. AND M. B. VISSCHER. This Journal **125**: 461, 1939.
- (15) KATZ, L. N. Blood, heart and circulation. Publ. of Am. Assoc. Adv. Sci. no. 13, Science Press, 1940, p. 194.
- (16) KATZ, L. N., W. WISE AND K. JOCHIM. This Journal **143**: 479, 1945.
- (17) KATZ, L. N., W. WISE AND K. JOCHIM. This Journal **143**: 495, 1945.
- (18) LANDOWNE, M. AND L. N. KATZ. Medical Physics. Year Book Publishers, 1944, p. 578.

ABSENCE OF RAPID DETERIORATION IN MODERATELY ACTIVE YOUNG MEN ON A RESTRICTED INTAKE OF B-COMPLEX VITAMINS AND ANIMAL PROTEIN

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It has been variously claimed that when a diet low in the B-complex vitamins is fed, there is rapid physical and psychic deterioration, and conversely, that such deterioration is completely absent. This discrepancy might be explained by the following: (1) primary reliance upon the appearance of subjective changes; (2) the incorrect association of causes and effects when one or more changes are made in the conditions of the experiment; (3) marked differences in the types of subjects involved; (4) the failure to consider that a lowered intake of a single nutrient may cause effects not directly related to that nutrient but mediated through an impaired "balance" of nutrients; (5) the failure to consider that the general character of the diet influences the effects of lowered intake of a single nutrient; and (6) the tendency to consider group effects without sufficient acknowledgment of the individual's biologic entity, with a consequence of difference in individual responses to a similar dietary circumstance. While there are undoubtedly other factors, the foregoing suffice to indicate that results which appear to be widely different may yet be capable of a harmonious interpretation if it were possible to interpret the effects of heterogeneity in experimental situations. Because such is not always possible, data in any scientific field may seem contradictory until the pattern is completed.

The maintenance of physical and mental efficiency is of great importance generally, and particularly so in military operations. If dietary inadequacy for short periods of time may cause adverse effects, then this information is of top military and medical significance. In attempting to investigate the relationship of these two groups of factors, it is important to recall that, in actual practice, dietary inadequacies are rarely single. They occur in groups, and the reversal of their effects usually depends upon the adequate provision of all of the required nutrients. The one outstanding exception to this concept is that of a caloric inadequacy.

One group of dietary inadequacies which might be encountered involves the following: low animal protein, thiamine, riboflavin, niacin. A diet of this char-

acter often contains less than average levels of the "lesser-known" B-complex factors such as folic acid, pantothenic acid, pyridoxine, biotin, etc. It is the purpose of this report to describe the results observed during a short term restricted intake of these nutrients by seven volunteer subjects. Certain minerals were incidentally consumed at levels lower than those recommended for optimal nutrition.

PROGRAM. *General.* Frequent measurements of physical efficiency and psychomotor response were made on seven normal young men (volunteers aged 23 to 28), subsisting for twelve weeks on a carefully controlled, adequate intake of nutrients in a normal diet. At the end of twelve weeks, it was considered that the period of learning of the various testing techniques had been passed, and that a base line had been reached with which any effects caused by an experimental diet could be validly compared. The subjects carried on their normal occupations of attending classes in the university, doing laboratory or clerical work, playing basketball, going for walks and hikes, etc. No attempt was made to control the type or extent of activity, it having been decided that the maintenance of the individual's body weight would be accepted (with certain possible reservations) as the criterion of caloric adequacy. Meals were prepared under the supervision of trained dietitians, and served at regular hours in a pleasant environment.

Urine was collected each week over a period of four consecutive days, the amber-colored one-gallon containers for which were kept in a refrigerator at all times. At the end of each day the total volume and a one-tenth aliquot were measured. The aliquot of each daily sample was transferred to a sample bottle which was kept in a deep-freeze unit. Over the same four-day period each week, feces were collected into a tared one-quart jar which was kept in a deep-freeze unit at all times. Shortly after the end of the four-day period the feces were thawed and homogenized, daily average excretion computed, and aliquots taken for assay. In some cases, fecal collection was continued slightly beyond the four-day period to allow the collection of three complete stools. Routine blood chemistry was done occasionally. Food was analyzed once a week.

Diet. The nutritional content of the normal and experimental diets is shown in table 1. The experimental diet contained the types of foods frequently encountered in everyday feeding in some parts of the country, as may be judged by the sample menu shown in table 2. In addition to the low B-complex content, corn was utilized at the level of 27 per cent of the total calories of the diet, and the level of animal protein was very low. Krehl et al. (1 and 1a) and Wintrobe et al. (2). There were only occasional returns of unconsumed foods, and it may be considered that the diet as analyzed represented the actual intake.

METHODS. The methods used for measuring change in (a) physical efficiency and (b) psychomotor response were as follows and have been discussed in detail elsewhere (3). (a) *Physical Efficiency.* (1) *Electrodynamic brake bicycle ergometer.* A stationary bicycle frame arranged for a variable, electrically controlled resistance against rotation of the pedals. A double work period to leg muscle exhaustion was used, separated by a 10-minute rest on a cot. Measure-

TABLE 1
Nutritional content of the normal and experimental diets

NUTRIENTS		NORMAL DIET (12 WEEKS)	EXPERIMENTAL DIET (5 WEEKS)
Calories.....		3170	3300
Protein.....	gm.	70	48*
(1-Tryptophane).....	mgm.	750-900	210-300
Carbohydrate.....	gm.	330	380
Fat.....	gm.	174	175
Calcium.....	gm.	0.86	0.20
Phosphorus.....	gm.	1.26	0.58
Iron.....	mgm.	15.5	12.0
Thiamine.....	mgm.	1.44	0.54
Riboflavin.....	mgm.	1.84	0.34
Niacin.....	mgm.	15.6	6.8
Biotin.....	mcg.	44	24
Folic acid.....	mcg.	64	26
Pantothenic acid.....	mgm.	4.7	1.3
Para-aminobenzoic acid.....	mgm.	0.21	0.30
Pyridoxine.....	mgm.	1.76	1.16
Ascorbic acid.....	mgm.	105	90
Vitamin A.....	I.U.	7400	22000†

* Approximately 94 per cent non-animal protein.

† Mostly b-carotene.

TABLE 2
Sample menu of experimental diet

Breakfast	Dinner
Applesauce	Baked hominy grits
Fried cornmeal mush	Hot beets
Fried salt pork	Perfection salad
Karo syrup with maple	Plain cornmeal muffins
Oleo	Cranberry sauce
	Oleo
	Apple betty
Lunch	Bedtime
Baked spaghetti casserole	Cornmeal muffins
with corn muffin crumb topping	Grape jelly
Green beans	Lemon crystals
Carrots	Sugar
Pickle relish	
Plain cornmeal muffins	
Oleo	
Pear halves	

ments were made in terms of resting and recovery pulse rate and duration of each ride.

(2) *Treadmill*. A standard run with a belt speed of 6 m.p.h. up a 10 per cent grade. Two runs of 4 minutes each were used with an intervening 10-minute rest. Measurement was made in terms of pulse recovery rate. Data were also obtained on resting pulse rates.

(3) *Harvard step test*. A 20-inch step with 30 up-down cycles completed per minute for 5 minutes. Measurements of pulse rate and blood pressure recovery, compared to resting reading were made.

(b) *Psychomotor response*. (1) *Johnson code test*. A mentation measurement using code letter substitutions. The time required to complete a given number of problems was recorded, as were the number of errors.

(2) *Hand steadiness test*. A device for measuring number and duration of contacts made between a perforated plate and a stylus held in subject's outstretched hand, movements being recorded in all three dimensions.

(3) *Single dimension pursuit test*. An instrument developed by the School of Aviation Medicine, Randolph Field, Texas, which records the total time an artificial moving horizon is kept in line with the reference points of the instrument.

(4) *Rotary pursuit maze*. A maze pattern cut in a rotating metal drum which is traced by the subject with a stylus, arranged to record number and duration of contacts for a standard period of time.

(5) *Pursuit meter*. A miniature airplane device which records errors in correcting positions of the airplane in three co-ordinates, involving separate use of both hands and the right foot.

(6) *Ataxiameter*. A device for recording body sway in two dimensions for a standard period of time.

The biochemical methods used for analysis of urine, food and feces were as follows, with appropriate modifications being made in the preparation for assay, according to the type of material:

(a) *Thiamine*. The method of Hennessy and Cerecedo (4) as modified by Friedemann and Kmiecik (5) (fluorometric).

(b) *Riboflavin*. The method of Ferrebee (6) with slight modification (fluorometric).

(c) *Folic acid*. The method of Teply and Elvehjem (7) (microbiologic).

(d) *Pantothenic acid*. The method of Hoag et al. (8) (microbiologic). Crystalline vitamin solutions were substituted for the yeast and vitab mixtures.

(e) *Biotin*. Originally by the method of Shull and Peterson (9), (microbiologic). Later the method of Teply and Elvehjem for folic acid was used for biotin assays.

(f) *P-aminobenzoic acid*. With *Neurospora crassa* by using a method based on that of Thompson et al. (10) and that of Stokes et al. (11) (microbiologic).

(g) *Pyridoxine*. A method based on that of Stokes and co-workers using *Neurospora sitophila* (11) (microbiologic).

(h) *Nicotinic acid*. The method of Krehl and co-workers (12) (microbiologic).

(i) *N¹-methylnicotinamide*. A modification of Najjar's method (13) (colorimetric).

(j) *Tryptophane*. A method based on that of Wooley and Sebrell (14) (microbiologic).

Feces and food samples were prepared for folic acid, pantothenic acid and biotin assays by the procedures outlined by the University of Texas workers (15). The biotin value for food obtained by the enzyme method was checked occasionally by autoclaving a duplicate sample with HCl for $\frac{1}{2}$ -hour, neutralizing and assaying.

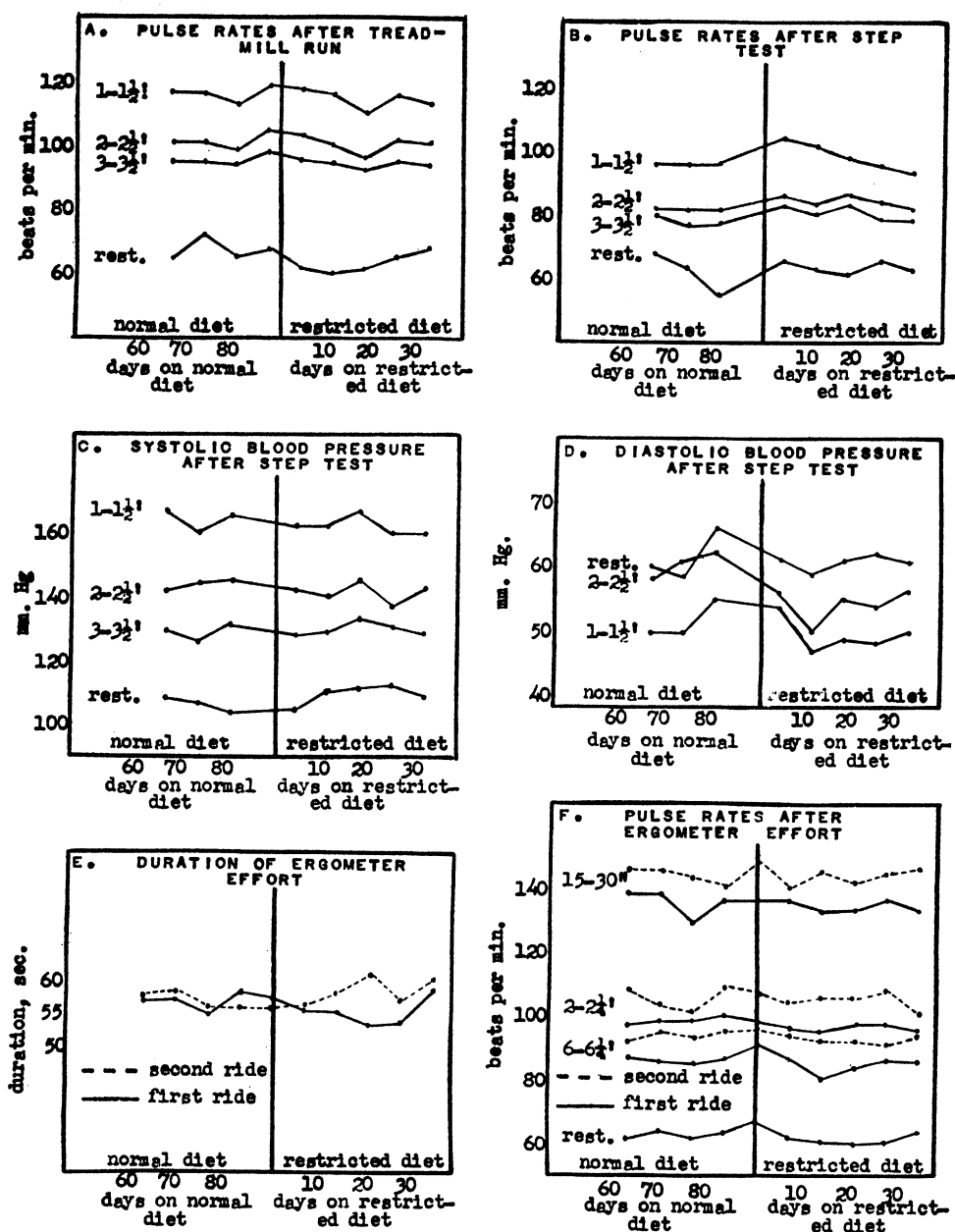


Fig. 1. Group average physical test results on normal and restricted diets.

RESULTS. The various group average scores on the physical and psychomotor tests are shown in figures 1 and 2, respectively. It will be observed that no significant change occurred during the experimental period of five weeks, as compared with the readings immediately preceding the beginning of the experimental diet, i.e., on the last three to four weeks on the normal control diet.

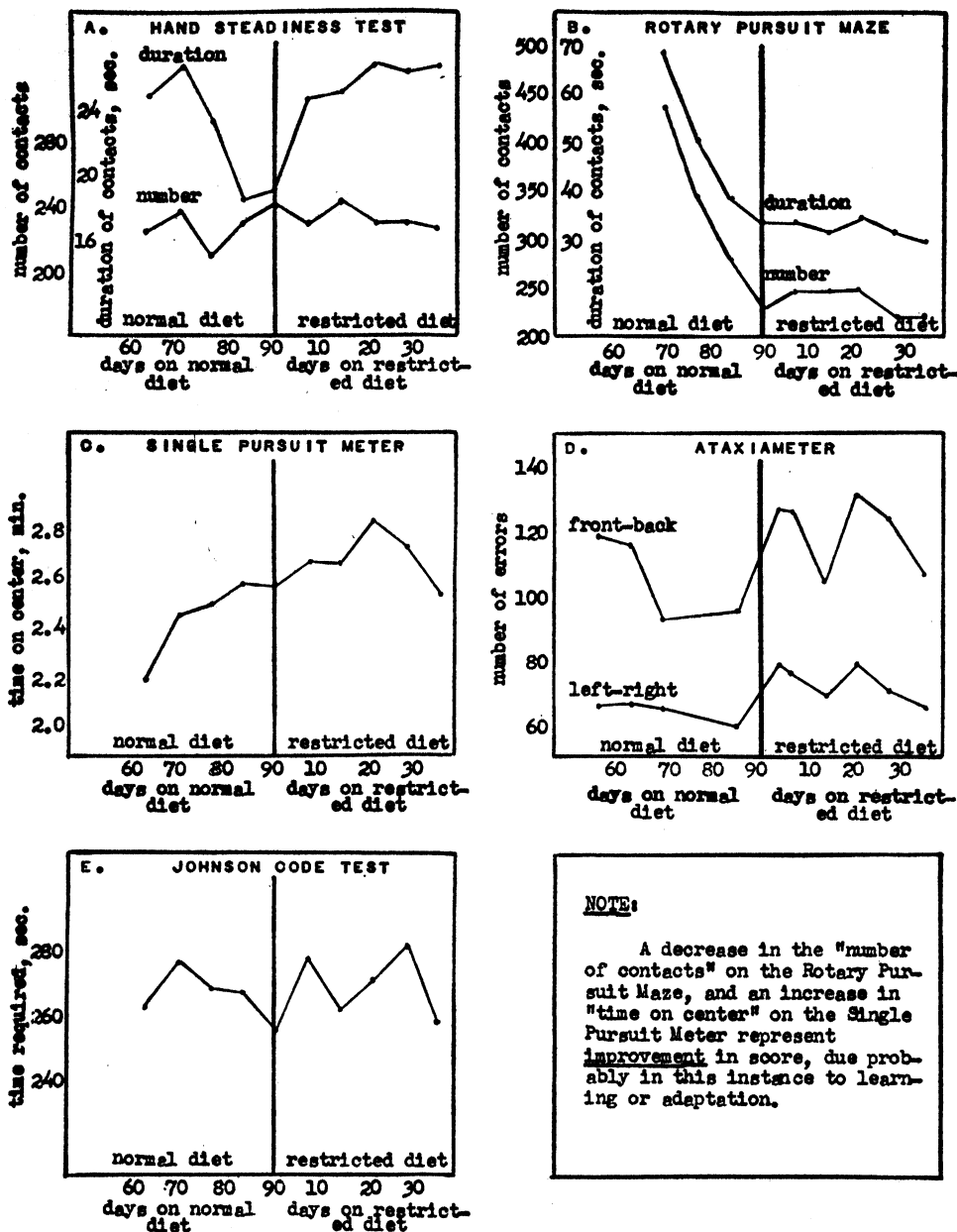
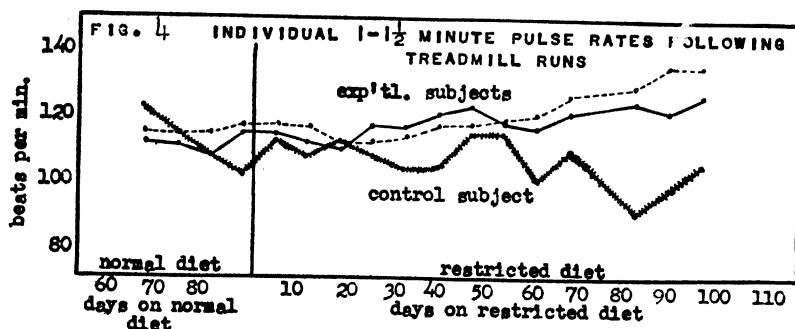
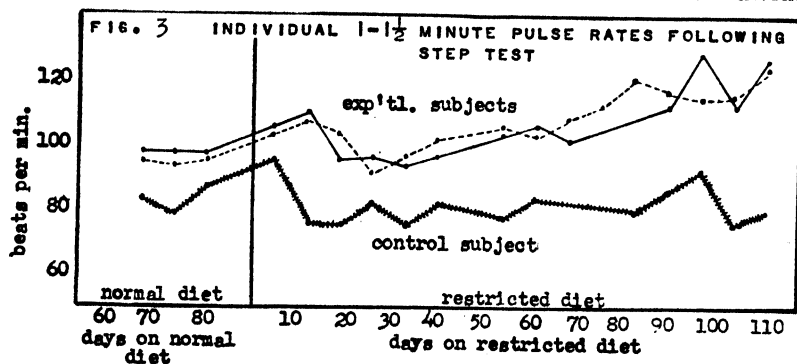


Fig. 2. Group average psychomotor test results on normal and restricted diets.

These latter readings are used for comparison because they presumably represented the normal average performance after the plateau of adaptation had been reached.

Although none of the *group* responses showed any change of significance, we cannot be absolutely certain that a change in some of the individual responses did

not occur. While we were not particularly impressed at the end of the five-week period that the individual responses showed any significant difference from the individual control levels previously determined, we have come to question, in retrospect, whether or not early changes actually might have begun. Figure 3 presents the individual graphs of the pulse rates in two of the subjects following the step test at the end of an additional ten weeks on the diet (i.e., total of fifteen weeks). At this time, definite changes¹ were observed in both group average and individual responses. If one examines the data for some of the individuals as



observed towards the end of the five-week period here reported upon, it might be said that the trend, which eventually became established later, had begun at the end of the first five weeks and then progressed with the dietary restriction through the fifteenth week. In contrast, the longer term graphs for two control subjects (two of the seven subjects supplemented by crystalline vitamins and animal protein immediately after the completion of the five-week period reported upon) did not show any such trends. While, therefore, we have interpreted the general group average results obtained at the end of a five-week period as showing no conclusive or rapid deteriorative change as determined by the tests used, we wish to draw attention to the possibility that some effect may have occurred in the cases of the two individuals cited. A similar situation arises in the case of the individual treadmill data (fig. 4). It must be pointed out, however, that even

¹ In order to be proven to be nutritional in origin, these changes should be reversible by appropriate supplementation to be carried out later.

when the individual changes are considered, the scores obtained at the end of the five-week period still indicated excellent physical conditions as compared with scores ordinarily obtained on cross-sections of well-fed soldiers or young men chosen at random. There was no evidence of group or individual deterioration in the psychomotor tests.

Clinical and Subjective Symptoms. Throughout the five-week period of ingestion of the experimental diet, there were no persistent subjective or objective findings which could be attributed to lack of essential nutrients. The sporadic appearance of headache and an occasional feeling of slight nausea have a highly questionable significance, because of their vagueness, rapid remission without therapy, lack of obviously debilitating effect, and absence of uniformity in any one subject or from one subject to another. There was no decrease in body weight at the end of the five-week period as compared with the stabilized body weight attained during the control period.

Biochemical Data. The biochemical data are presented in table 3. It is shown that the twenty-four hour group average excretion of the majority of the vitamins decreased rapidly in the urine, and in the cases of thiamine, riboflavin and the F_2 factor, the drop was immediate and very marked. The load test response for those vitamins similarly underwent a pronounced decrease. A positive nitrogen balance was maintained.

It is of particular interest to note that the group average fecal excretion of none of the vitamins determined (including the lesser-known B-complex vitamins) showed any decrease whatever during the five-week experimental period. The authors will describe in detail in a separate communication, these biochemical data showing a high fecal content of vitamins in the presence of a relatively low dietary intake and urinary output. In general, the biochemical data reflected the change in diet, with the striking exception of the feces.

SUMMARY

Following a twelve-week period on a normal diet, seven normal young men were fed for five weeks a diet containing 0.16 mgm., 0.11 mgm. and 2.1 mgm. of thiamine, riboflavin and niacin, respectively, per 1000 calories of food consumed. The intake of the various lesser-known B-complex vitamins (para-aminobenzoic acid excepted) ranged from 28 per cent to 66 per cent of that in the normal diet. The intake of protein was 48 grams, of which approximately 94 per cent was non-animal in type. Corn comprised 27 per cent of the total calories of the diet. Most of these dietary changes were reflected in the urinary excretion levels within one week. No decrease occurred at any time in the fecal content of the vitamins, however. No effect was observed in the group average physical or psychomotor responses. Examination in retrospect of certain of the individual measurements of physical efficiency indicate the possibility that in two individuals, slight beginning changes may have occurred in that category, but not in any of the tests used for measuring psychomotor function. These individual changes were not statistically significant for the five-week period, but do, however, comprise part of a statistically significant change observed at the end of fifteen weeks of the experimental diet.

TABLE 3

Group average composition of body fluids in experimental period compared with control period

	AVERAGE* AT END OF CONTROL PERIOD	WEEKS ON THE EXPERIMENTAL DIET				
		1	2	3	4	5
24 hour urine excretion						
Thiamine, mgm.....	0.24	0.08	0.04	—	0.02	0.02
Riboflavin, mgm.....	0.79	0.35	0.22	—	0.18	0.19
Niacin, niacinamide, nico- tinuric acid, mgm.....	1.0	1.0	0.9	0.8	—	—
F ₂ , mgm.....	3.9	2.2	2.0	1.0	0.8	0.5
Folic acid, mcg.....	4.6	—	—	5.2	—	5.7
Pantothenic acid, mgm.....	3.1	2.9	1.9	1.9	1.9	1.8
Para-aminobenzoic acid, mgm.....	0.19	0.15	0.26	0.18	0.16	0.21
Pyridoxine, mgm.....	0.40	0.25	0.27	0.26	0.17	0.19
Biotin, mcg.....	34	26	15	14	18	19
l-Tryptophane, mgm.....	13.0	11.8	11.4	14.5	14.1	12.5
Uric acid, gm.....	0.55	—	—	—	0.63	0.50
Creatinine, gm.....	1.97	1.69	—	—	1.73	1.72
Nitrogen, gm.....	9.45	6.34	5.12	4.84	4.56	4.61
24-hour feces excretion						
Thiamine, mgm.....	0.58	0.53	0.64	—	0.63	—
Riboflavin, mgm.....	1.00	0.98	1.10	—	1.1	—
Niacin, niacinamide, nico- tinuric acid, mgm.....	3.4	4.4	5.8	6.1	6.5	—
Folic acid, mgm.....	0.30	0.41	0.38	0.33	0.45	—
Pantothenic acid, mgm.....	1.9	3.2	3.2	3.8	3.5	3.8
Para-aminobenzoic acid, mgm.....	0.25	0.31	0.34	0.29	0.28	0.30
Pyridoxine, mgm.....	0.30	0.36	0.40	0.38	0.35	0.27
Biotin, mgm.....	0.12	0.15	0.14	0.14	0.13	0.13
l-Tryptophane, mgm.....	—	7.9	5.6	13.6	11.0	—
Nitrogen, gm.....	1.34	1.74	1.42	1.37	1.12	—
Load test response†						
Thiamine, mcg. per 4 hr.....	123 (96)	—	—	—	—	64 (61)
Riboflavin, mcg. per 4 hr.....	245 (139)	—	—	—	—	103 (67)
F ₂ , mgm. per 4 hr.....	3.12 (1.56)	—	—	—	—	1.43 (1.37)
Blood						
Serum protein						
Total: gm./100 ml.	6.6	6.4	6.4	—	—	6.5
albumin, gm./100 ml.	4.8	—	4.5	—	—	4.6
globulin, gm./100 ml.	1.8	—	1.9	—	—	1.9
Hemoglobin, gm./100 ml.	15.2	—	14.6	—	—	15.3
Red blood cell, million/mm. ³	5.18	—	5.07	—	—	5.28

* Last 3 tests during control period except in cases where fewer than 3 tests were done in the last half of the control period.

† Values in parentheses represent excretion in excess of 4 hour fasting excretion; 0.5 mgm. thiamine, 0.5 mgm. riboflavin, and 10.0 mgm. niacinamide used intravenously.

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REFERENCES

- (1) KREHL, W. A., L. J. TEPLY AND C. A. ELVEHJEM. *Science* **101**: 2620, 1945.
- (1a) KREHL, W. A., L. J. TEPLY, P. S. SARMA AND C. A. ELVEHJEM. *Science* **101**: 2628, 1945.
- (2) WINTROBE, M. M., H. J. STEIN, R. H. FOLLIS, JR. AND S. HUMPHREYS. *J. Nutrition* **30**: 6, 1945.
- (3) COGSWELL, R. C., C. R. HENDERSON, G. H. BERRYMAN, ET AL. *This Journal* **146**: 422, 1946.
- (4) HENNESSY, D. J. AND L. R. CERECEDO. *J. Am. Chem. Soc.* **61**: 179, 1939.
- (5) FRIEDEMANN, T. E. AND T. C. KMIECIAK. *J. Lab. Clin. Med.* **28**: 1262, 1943.
- (6) FERREBEE, J. W. *J. Clin. Investigation* **19**: 251, 1940.
- (7) TEPLY, L. J. AND C. A. ELVEHJEM. *J. Biol. Chem.* **157**: 303, 1945.
- (8) HOAG, E. H., H. P. SARETT AND V. H. CHELDELIN. *Ind. Eng. Chem. Anal. Ed.* **17**: 60, 1945.
- (9) SHULL, G. M. AND W. H. PETERSON. *J. Biol. Chem.* **151**: 201, 1943.
- (10) THOMPSON, R. C., E. R. ISBELL AND H. K. MITCHELL. *J. Biol. Chem.* **148**: 281, 1943.
- (11) STOKES, J. L., A. LARSON, C. R. WOODWARD, JR. AND J. W. FOSTER. *J. Biol. Chem.* **150**: 17, 1943.
- (12) KREHL, W. A., F. M. STRONG AND C. A. ELVEHJEM. *Ind. Eng. Chem. Anal. Ed.* **15**: 471, 1943.
- (13) NAJJAR, V. A. *Bull. Johns Hopkins Hosp.* **74**: 392, 1944.
- (14) WOOLEY, J. G. AND W. H. SEBRELL. *J. Biol. Chem.* **157**: 141, 1945.
- (15) CHELDELIN, V. H., M. A. EPPRIGHT, E. E. SNELL AND B. M. GUIRARD. *University of Texas Publication* **4237**: 15, 1942.

THE DISTRIBUTION OF WATER AND FAT IN THE SKIN AND MUSCLE OF THE DOG DURING CHRONIC AND ACUTE DEHYDRATION

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Considerable variation in both fat and water content of tissues and organs occurs under certain experimental conditions. High fat diet has been shown by Haldi et al. (1940, 1941) and Wynn and Haldi (1944) to result in little change in the fat and water content of the entire body, but the percentage of water in the integument appeared to decrease accompanied by an increase in the fat content of this organ. High carbohydrate diets produced similar but less marked changes (Haldi et al., 1940, 1941). They also reported (1944) that in animals fed vitamin deficient diets, compared with controls having a correspondingly low caloric intake, the water content of the skin fluctuated with an increase in the females and a decrease in the males. The water content of the whole body did not differ in either sex. When the caloric intake of the controls of either sex was adequate, the water content of their skin and entire body was lower than in the vitamin deficient animals. With reference to the integument they concluded that under various experimental conditions the water content varies inversely with the fat content (1941). It is apparent that low food and water intake results in greater storage of water by the skin than occurs during a period of favorable nutrition.

Skelton (1927) showed that rapid dehydration was accompanied by a decrease in water content of the skin and musculature. Ricca et al. (1945) observed that skin and striated musculature, during acute traumatic shock, lost considerable water. These results tend to show that under conditions of rapid dehydration which would not be expected to change the fat content of tissues appreciably there is a decrease in water content of the integument and muscles.

In the course of a study of the effects of chronic dehydration by inanition and water deprivation and acute dehydration resulting from mild hemorrhage, or injection of sucrose, changes in fat and water content of the skin and muscle were obtained. This investigation is an attempt to determine whether in the dog conditions of acute and chronic dehydration induce significant variation in the water content of the skin and musculature accompanying the changes in the amount of fat.

MATERIAL AND METHODS. Samples of skin from the back, fore and hind legs, and from the striated muscles in these same areas were obtained from dogs before and after a period of chronic dehydration by water deprivation and fasting for 4 to 6 days. All operations were carried out under pentobarbital anesthesia. At no time did the animals show any untoward symptoms.

Acute dehydration by hemorrhage of approximately 12 grams of blood per kilogram of body weight or the intravenous injection of 100 cc. of 50 per cent sucrose was also carried out under pentobarbital anesthesia. Normal samples of skin and muscle were obtained before the experimental dehydration procedure and the second set of samples was taken approximately 3 hours later.

Each sample was placed immediately into a dry weighing bottle, weighed, and then cut into small pieces and placed in a glass stoppered vial containing 25 cc. of purified ethyl alcohol. The material was dried in a constant temperature oven at $103 \pm 1^\circ\text{C}$. The fat was removed by ether extraction after which the material was again dried to obtain the amount of fat-free solid material present.

RESULTS. Chronic dehydration was accompanied by a decrease in the fat content of the skin from an average of 18 per cent in normal samples to 14 per

TABLE 1
The water content of the skin during chronic and acute dehydration

DOG NO.	CHRONIC DEHYDRATION				DOG NO.	ACUTE DEHYDRATION			
	Whole tissue		Fat-free tissue			Whole tissue		Fat-free tissue	
	Per cent water in control	Per cent water after dehydration	Per cent water in control	Per cent water after dehydration		Per cent water in control	Per cent water after dehydration	Per cent water in control	Per cent water after dehydration
1	75.9	65.8	81.5	70.2	4	62.0	59.7	73.5	72.1
3	82.3	71.4	83.7	71.7	5	64.3	61.6	71.8	74.1
4	60.2	70.5	75.4	74.2	6	72.4	69.7	73.1	69.8
5	61.0	54.7	76.3	74.6	11	54.4	56.4	75.3	72.4
6	71.6	72.1	76.6	75.3	14	59.9	49.0	74.1	68.1
7	44.2	50.8	75.5	77.8	15	62.0	63.4	76.0	72.3
8	57.0	51.8	75.4	71.7					
10	57.3	51.5	75.6	67.7					
Average...	63.7	61.8	77.5	72.9		62.5	60.0	74.0	71.5

cent in samples obtained at the height of dehydration. The decrease was greater in obese dogs. The percentage of fat in samples of skin obtained after hemorrhage or sucrose injection deviated very little from the normal. The fat content of the musculature averaged less than 5 per cent and decreased less than 0.5 per cent accompanying both chronic and acute dehydration.

The water content of the skin, when determinations were made on samples from which the fat had not been removed, apparently decreased 1.5 per cent in the animals dehydrated by fasting (table 1). However, during acute dehydration the water content decreased 2.5 per cent. When the percentage of water per gram of tissue is calculated upon fat-free tissue the loss of water by the integument during chronic dehydration is greater than the water lost by the skin during acute dehydration. Chronic dehydration was accompanied by a 4.6 per cent decrease in water content in the fat-free tissue as compared to a decrease of 2.5 per cent in acutely dehydrated dogs. The decrease in water

content after hemorrhage or sucrose injection was the same when calculated for whole or fat-free samples.

The water content of the musculature appears to be considerably higher than that of the integument with an average of approximately 76 per cent for muscle (table 2) as compared to 63 per cent for skin (table 1). In fat-free tissue, however, the water content of normal skin and muscle samples differs by less than 5 per cent. This is due to the fact that in the integument are large amounts of adipose tissue which are water free. The average loss of water from the musculature during chronic dehydration appears to be 1.5 per cent as compared to a loss of 0.7 per cent during acute dehydration. However, when based on the

TABLE 2

The water content of the skeletal musculature during chronic and acute dehydration

DOG NO.	CHRONIC DEHYDRATION				DOG NO.	ACUTE DEHYDRATION			
	Whole tissue		Fat-free tissue			Whole tissue		Fat-free tissue	
	Per cent water in control	Per cent water after dehydration	Per cent water in control	Per cent water after dehydration		Per cent water in control	Per cent water after dehydration	Per cent water in control	Per cent water after dehydration
1	77.4	78.0	79.4	79.4	4	74.4	73.6	76.9	77.3
2	76.3	76.3	78.0	78.0	5	78.5	76.7	80.5	78.7
3	79.1	76.4	80.9	77.4	6	77.8	76.3	79.3	77.4
4	76.6	73.9	78.4	75.6	11	75.9	75.1	78.9	77.8
5	80.1	77.0	82.2	79.4	14	78.7	76.8	81.5	80.4
6	76.1	77.9	79.8	79.4	15	75.0	77.3	82.1	80.0
7	69.3	68.6	79.8	79.2					
8	72.4	72.3	80.3	79.5					
10	77.5	72.8	80.6	76.1					
13	79.3	76.1	80.3	78.2					
Average...	76.4	74.9	80.0	78.4		76.7	76.0	79.7	78.6

fat-free samples the water content of the musculature decreased 1.6 per cent during chronic dehydration and 1.1 per cent during acute dehydration.

A comparison was made of the changes in the water content of the skin of those dogs (nos. 4, 5, 7, 8, 10, 11, 14) considered obese because samples of the normal skin contained more than 10 per cent fat with the animals from whom samples of low fat content were obtained. The water content of the normal skin as determined in fat-free samples of the obese animals was 74.9 per cent as compared with the average of 76 per cent for all the animals under normal conditions. The percentage of water in the skin of the animals with a low fat content in the skin was 78 per cent.

The water in the skin of obese animals appeared to increase during chronic dehydration from an average of 57 per cent to 61 per cent. When determinations were based upon fat-free samples, however, chronic dehydration resulted in a reduction of approximately 2 per cent in the water content of the skin in fat

dogs. The lean animals showed a decrease in water content from 76 per cent in the control samples to 70 per cent after fasting. The percentage of water in the skin decreased approximately 8 per cent when determinations were made of fat-free tissue. Sex appeared to be of no significance; however, the paucity of male animals did not permit any generalizations. The fat content of the striated muscles did not vary significantly in any of the animals, being less than 5 per cent in all but a few samples in either acutely or chronically dehydrated dogs.

DISCUSSION. It is apparent that general obesity may be correlated with the water content of the integument and the whole body. The data of Harrison et al. (1936) when recalculated on the basis of fat-free tissue suggest that in dog and monkey the water content of the whole body is slightly greater in lean animals. The data of Haldi et al. (1941) recalculated on the basis of fat-free samples indicate that this occurs in rats and appears to be correlated with sex, in that females with a higher fat content of the integument have a slightly lower water content than males. The data presented in this paper suggest that conditions, hormonal perhaps, producing obesity in dogs tend to lower the water content of the non-adipose constituents of the integument. However, when comparisons based upon fat-free tissue are made of the water content of animals on high fat diets, the data of Wynn and Haldi (1944) do not seem to indicate a decrease in the percentage of water in the integument or the body as a whole. Rapid increase in the fat content of the skin accompanying high fat or carbohydrate diets results in an apparent rather than actual decrease in the water.

Vitamin deficiency in the rat accompanied by low caloric intake or low caloric diet alone resulted in higher water content of skin than when an adequate diet was fed (Haldi et al., 1944). Although no data on fat content were given, it seems likely, in view of the changes occurring during dehydration in the dog, that low caloric diets were accompanied by a depletion of stored fat and an apparent, rather than actual, increase in the water of the skin.

Data on body water should be calculated on the basis of fat-free constituents, since differences in adipose tissue mask real variations in water content. Failure to do this has accounted for many inconsistencies in the literature in this field.

SUMMARY

Chronic dehydration in the dog by withholding food and water was accompanied by a decrease in fat of 4 per cent in skin and less than 0.5 per cent in skeletal muscle. Acute dehydration by mild hemorrhage or sucrose injection resulted in no significant change in fat.

Data on water content of normal and dehydrated tissues were more consistent and significant when calculations were based upon fat-free samples. Normal skin and muscle then differed less than 5 per cent in water content. The percentage loss of water during dehydration was greater for skin than for muscle.

High fat content was correlated with a lower percentage of water in the fat-free constituents of the skin of normal dogs. During chronic dehydration, the loss of water by skin was less for obese dogs than for lean dogs.

Since normal or experimentally induced variations in fat content result in apparent differences in the percentage of water of whole tissue, it is concluded that comparisons of body water should be based upon fat-free samples.

REFERENCES

- HALDI, J. AND G. GIDDINGS. This Journal **128**: 537, 1940.
HALDI, J., G. GIDDINGS AND W. WYNN. This Journal **135**: 392, 1941.
This Journal **141**: 83, 1944.
HARRISON, H. E., D. C. DARROW AND H. YANNET. J. Biol. Chem. **113**: 515, 1936.
RICCA, R. A., K. FINK, L. T. STEADMAN AND S. L. WARREN. J. Clin. Investigation **24**: 140, 1945.
SKELTON, H. Arch. Int. Med. **40**: 140, 1927.
WYNN, W. AND J. HALDI. This Journal **142**: 508, 1944.

ARTERIAL OXYGEN PRESSURE DURING PURE O₂ BREATHING

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Much work has been done on gas diffusion through the lung since Pfüger et al. (1871) concluded from their experiments that the exchange between blood and alveolar gases follows the law of diffusion. Pfüger's conclusion, challenged by Ludwig, was later confirmed by Krogh, a pupil of Ludwig. This view was accepted, with some limitations, even by Haldane, long a partisan of lung oxygen secretion.

The diffusion theory implies that in all cases the partial pressure of oxygen (pO₂) in the arterial blood should be equal or lower than the corresponding pressure in the alveoli. The difference in oxygen pressure (Δ pO₂) between alveolar air and arterial blood has been extensively studied. However, there is a lack of agreement on its measurement; gradient differences as great as 30 mm. Hg and as small as 1 mm. have been reported in the literature.²

Recently Roughton, Darling, and Root (1944) concluded that the tonometer method gives too low values for the arterial pO₂ because the oxygen capacity obtained is erroneously high. Correction for this error raises the average percentage saturation of arterial blood of a normal man at sea level from 95 to 97, and the arterial pO₂ calculated from these figures and the blood dissociation curves comes out to 100 mm. Hg. This is supported by the work of Comroe and Dripps (1944) and Drabkin et al. (1944) who, with different techniques, did not find appreciable O₂ pressure differences between alveoli and blood.

The alveolar-arterial equilibrium when breathing pure oxygen has not attracted much attention. Most of the work done on this subject is open to criticism on account of the method used (1, 6, 7, 8). Berggren³ (1942), quoted by Comroe et al., employing the polarographic method of determining the arterial pO₂ of subjects breathing 100 per cent O₂, found that there was an average difference of 11 mm. Hg between alveolar air and arterial blood. In 9 patients confined to bed the difference increased to 41 mm. Hg.

Before studying the gas equilibrium between alveolar air and arterial blood during oxygen breathing in cases of respiratory disease we found it desirable to know first the equilibrium conditions in normal individuals.

METHODS. Two methods were employed: In method I oxygen pressure was calculated from the value of the dissolved oxygen in plasma obtained by centrifuging anaerobically arterial blood in a specially devised tube. In method II the dissolved oxygen, and therefore the corresponding oxygen pressure, was

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² For detailed bibliography see Comroe et al. and Roughton et al.

³ Unfortunately we were unable to get the original paper.

obtained by subtracting from the total arterial oxygen content the oxygen capacity of the hemoglobin, determined by equilibrating a sample of the same blood in a tonometer, at a known oxygen pressure.

Method I. It is a basic condition of the method that centrifugation must be done without any loss of gas. Since centrifugation under oil and a layer of solid paraffin proved to be inadequate we used the specially devised tube *S* (fig. 1) by means of which the centrifugation of blood over mercury was possible. The centrifuge tube, made of thick glass, has a length of about 8 cm., a diameter of 2.5 cm., and a capacity of 20 cc. At the top of the tube there is a one-way stopcock. A straight glass tube *b* goes from near the top to the bottom of the centrifuge tube. The upper end of tube *b*, is connected through a rubber tube to bulb *c*.

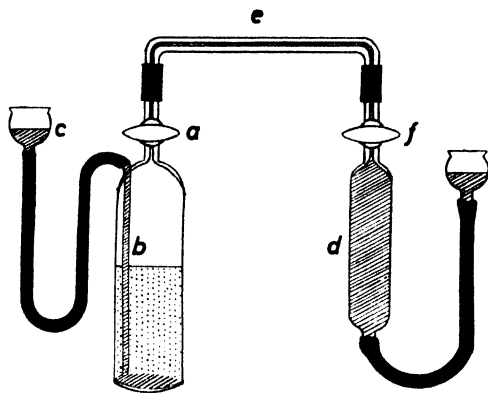


Fig. 1. The figure represents the complete apparatus ready for transferring the plasma separated by centrifugation.

On the left is the specially devised tube *S* and on the right a blood sampling tube. For further description see the text.

Tube *S* is used in the following way: Bulb *c*, the centrifuge tube, and the capillary of the stopcock *a*, are filled with mercury. With a syringe, blood is introduced through stopcock *a* approximately to the level represented in figure 1. The capillary of the stopcock is sealed with mercury, and fastened with a rubber band. The tube is placed in a 250 cc. centrifuge cup, the space between tube and cup is filled with cold water and cracked ice, and the whole left in the icebox for 10 minutes. This is done in order to diminish the O_2 consumption of the blood cells. Mercury bulb *c* is left in place to prevent air being sucked through tube *b*, when the liquid contracts due to the cooling. At the end of the 10 minutes, bulb *c* and the attached rubber tube are disconnected. By aspiration through a long needle, the mercury in tube *b* is brought to a level slightly higher than that of the mercury in the centrifuge tube. If the level of mercury is left at the top of tube *b*, the weight of the column during centrifugation will force blood through the stopcock. On the other hand, if the mercury level is too low in tube *b*, a negative pressure arises during centrifugation and some gas may escape from the blood.

After 5 minutes' centrifugation at 2000 r.p.m. tube *b* is filled with mercury and bulb "*c*" connected, trapping of air bubbles being avoided. The mercury seal of the stopcock is driven out by raising bulb *c* and the capillary cleared with a pipe cleaner and afterwards rinsed with a small amount of plasma.

Plasma is then transferred to a blood sampling tube. This is done as follows, figure 1: the sampling tube *b* and the glass capillary are filled with mercury. Raising mercury bulb *c* and opening stopcocks *a* and *f*, the plasma is transferred anaerobically into tube *d*. If jerky movements are avoided, most of the separated plasma can be transferred.

Plasma samples were analyzed for oxygen in the manometric apparatus of Van Slyke-Neill, Van Slyke pipettes of 1 cc. capacity being used, but in some cases, a 3 cc. anaerobic pipette was also employed. Analyses, done in duplicate, were carried out as usual, employing 6 cc. of a 0.5 per cent solution of lactic acid and a drop of caprylic alcohol instead of the ferricyanide solution.

The maximum difference between duplicates was 0.10 vol. per cent and the standard error of a single determination ± 0.039 vol. per cent and the standard error of a duplicate determination ± 0.027 .

The accuracy of the method was checked by equilibrating human plasma with different oxygen mixtures as follows: heparinized human plasma, 20–40 cc., was introduced in a tonometer of one liter capacity, filled with CO₂ and oxygen at the desired pressure and rotated in a water bath at 37° C. for 30 minutes. The plasma was then transferred *a*, directly to a sampling tube over mercury; *b*, to tube *S* and centrifuged 10 minutes at 2000 r.p.m.; *c*, to a syringe filled with oil (the oil was previously equilibrated with pure O₂) and then to a sampling tube over mercury. The oxygen content of each sample was determined as described above.

The pressure in the tonometer was measured at room temperature with a small mercury manometer, the barometric pressure and the temperature being read, and the composition of the gas determined in the Haldane-Boothby-Sandiford apparatus.

From these data the oxygen pressure was calculated as follows:

$$pO_2 = (B - w + pp) \frac{273 - 37}{273 - t} \times \frac{V - b_1 \times O_2\%}{V - b_2} =$$

Where

pO_2 : oxygen pressure in millimeters of Hg.

B: barometric pressure, in millimeters of Hg.

w: vapor pressure at room temperature, in millimeters of Hg.

pp: pressure in the tonometer (positive or negative), in millimeters of Hg.

V: capacity of the tonometer, in cubic centimeters.

*b*₁: residual plasma in the tonometer, in cubic centimeters.

*b*₂: cubic centimeters of plasma introduced in the tonometer.

Table 1 shows the results of the experiments. There are no appreciable differences between previously centrifuged and directly transferred plasma. As the blood from the patients was drawn under oil, it was considered desirable to

test for oxygen losses through the oil layer. Although the experimental data are not included no differences were observed whether the plasma was drained directly from the tonometer into the sampling tube or the oiled syringe used for transferring the blood from the tonometer to the sampling tube. In order to check for the possible loss of oxygen occurring during the manipulation with Van Slyke pipettes, the anaerobic pipette of 3 cc. described by Van Slyke and Neill (1924) was used in some cases, and no significant differences could be detected.

Sendroy, Dillon and Van Slyke (1934) found the oxygen solubility coefficient at 38° for horse plasma to be 0.0209. The coefficient was recalculated for 37° C., using chart 3 of their paper, in which α for whole blood is expressed as a function

TABLE 1

Oxygen content of human plasma equilibrated with oxygen in a tonometer during 30 minutes at 37°C. The theoretical values were calculated using the data of Sendroy, Dillon and Van Slyke (1934)

In the last two experiments the analyses were carried out both with a Van Slyke pipette of 1 cc. and with an anaerobic pipette of 3 cc. The results agree within the experimental error.

O ₂ PRESSURE IN TONOMETER	O ₂ CONTENT OF PLASMA		Δ FOUND—CALCULATED
	Found	Calculated	
mm. Hg	Vol. %	Vol. %	Vol. %
704	1.93	1.98	-0.05
709	1.95 1.97*	2.00	-0.05 0.03
706	1.98 2.01*	1.99	-0.01 +0.02

* Samples of the same plasma centrifuged in a tube S, 5 minutes at 2,000 r.p.m.

of temperature. The figure obtained for α plasma at 37° was 0.0214. Table 1 shows a comparison of the values of oxygen content found by us with those calculated using the absorption coefficient of Sendroy et al. at 37° C. As can be seen, the results agree within the experimental error.

Similar experiments were performed on whole blood; 25 cc. of heparinized human blood were introduced in a tonometer of one liter capacity, previously filled with oxygen and carbon dioxide at a pressure of about 700 and 40 mm. Hg respectively. Blood was equilibrated with the gas phase by rotating the tonometer for 30 minutes at 37° C. and then transferred to tube S and centrifuged 5 minutes at 2000 r.p.m. Further steps were as described above and the analyses were carried out on the separated plasma.

Values obtained were lower than those calculated using the data of Sendroy et al., as is shown by the following figures:

Plasma O ₂ content in vol. %	
Found	Calculated
1.70	2.00
1.95	2.23
1.68	1.96
1.77	1.97
1.93	2.08

We ascribed the differences as due to O₂ consumption by the blood cell phase. This possibility was tested by leaving the separated plasma in tube *S* at room temperature, together with the cell phase, and measuring the O₂ content of the plasma at various times. In one experiment the O₂ content of the plasma fell from 1.93 vol. per cent to 1.42 vol. per cent in 85 minutes at 22° C.; in the other, from 1.72 to 1.18 in 4 hours. Sodium cyanide did not prevent this O₂ consumption. On the other hand, no appreciable changes in the oxygen content of the separated plasma were observed when it was left for a few hours at room temperature. To minimize the oxygen consumption of the cells, working with whole blood it was found necessary to cool it and to carry out the complete procedure as rapidly as possible. Finally we adopted the technique described under method I, and the results obtained are summarized in table 2. It can be seen that the average values agree fairly well with the theoretically calculated ones. The scattering is greater than that expected from the analytical errors in the determination of O₂ plasma content, plus those involved in the determination of the pO₂ in the tonometer. We think that a small consumption of O₂ in some of the experiments could be admitted as one of the causes of such scattering.

Method II. Two portions of the same blood sample were equilibrated for 20 minutes at 37° C. and at oxygen pressures of about 200 and 700 mm. Hg respectively; enough CO₂ was added to produce a pCO₂ of about 40 mm. Hg. At the end of the equilibration time the blood was transferred over mercury and stored in the ice box. Duplicate analyses were made in the Van Slyke-Neill apparatus.

Methods for determination of the gas pressure in the tonometer have been described in detail previously. The absorption coefficients calculated from the data secured with such a technique are given in table 3 and are compared with those obtained by means of the formula α for blood at 37° C. = $0.0214 + 0.000108 \times (\text{vol. per cent capacity})$, which was obtained from the results given by Sendroy, Dillon and Van Slyke. The average values do not show good agreement with the coefficient obtained using the data of Sendroy et al., but the considerable scattering of the figures prevents us from drawing further conclusions.

EXPERIMENTAL PROCEDURE. The study of the arterial oxygen pressure during pure oxygen breathing was carried out in normal human subjects. The subjects in basal condition and after a 30 minute rest, inhaled the gas through a mouth-piece from a Tissot spirometer, during 15 to 30 minutes.

Respiratory minute volume and pulse rate were measured. At the end of the 15 to 30 minutes, breathing period, arterial blood was secured by puncture of the femoral artery, a 25 cc. sample being drawn under oil saturated with oxygen; clotting was avoided by adding 3 drops of heparin solution. While the subject was still breathing pure oxygen, two samples of expiratory alveolar air were

obtained. A portion of the blood was transferred at once into the chilled *S* tube, the remainder being divided between a common blood sampling tube for

TABLE 2

Oxygen dissolved in plasma (method I)

Human blood was equilibrated with oxygen in a tonometer for 30 minutes at 37°C., anaerobically centrifuged in an *S* tube 5 minutes at 2,000 revolutions, and the plasma transferred to a blood sampling tube over mercury. In the last four experiments blood was cooled with ice water as soon as tube *S* was filled.

The time elapsed between filling the *S* tube with blood and transferring the supernatant plasma never exceeded half an hour.

OXYGEN PRESSURE IN TONOMETER	O ₂ CONTENT OF PLASMA FOUND	O ₂ CONTENT OF PLASMA CALCULATED	Δ FOUND—CALCULATED
mm. Hg	Vol. %	Vol. %	Vol. %
724	1.97	2.04	-0.07
718	1.95	2.02	-0.07
744	2.00	2.09	-0.09
758	2.25	2.14	+0.11
749	2.16	2.11	+0.05
749	2.10	2.11	-0.01
748	2.10	2.11	-0.01
Average.....		-0.013 \pm 0.0302	

TABLE 3

Absorption coefficients for oxygen, found and calculated

Total oxygen content of two samples of the same blood equilibrated for 20 minutes at 37°C. at two different oxygen tensions; α is the absorption coefficient of the whole blood calculated using the data of Sendroy, Dillon and Van Slyke and using the figures of this table.

TONOMETER I		TONOMETER II		α	α
Gas phase O ₂ pressure	O ₂ content of the blood	Gas phase O ₂ pressure	O ₂ content of the blood	Found	Calculated
mm. Hg	cc per 100 cc.	mm. Hg	cc per 100 cc.		
195.4	20.34	737.8	22.31	0.0276	0.0235
174	20.04	723	21.69	0.0229	0.0234
193.8	21.76	709	23.86	0.0310	0.0236
200	20.37	707	21.92	0.0232	0.0235
193	22.30	719	24.09	0.0258	0.0237
Average.....				0.0261	0.0235

further O₂ analysis and a tonometer filled with a gas mixture giving a pCO₂ of 40 mm. Hg. and a pO₂ of 200 mm. Hg. Afterwards the tonometer was equilibrated at 37° C. for 10 minutes. The next steps were as described already under methods I and II.

The arterial pO_2 was calculated from the values of dissolved O_2 using the coefficient 0.0214 for plasma, and the formula: $0.0214 + 0.000108$ (vol. per cent capacity) for whole blood, as explained before.

In the first five experiments in normal subjects breathing oxygen, tube *S* was not cooled with iced water but only placed in the refrigerating coil of the ice box. Under these conditions, the O_2 consumption of the blood was found to be about 0.07 vol. per cent and therefore this figure was added to the oxygen content found in the arterial plasma of those subjects.

RESULTS. The results obtained in 8 normal subjects are summarized in table 4. In every case the O_2 pressure in arterial blood, calculated with method I, was

TABLE 4

Oxygen pressure in the arterial blood of normal subjects breathing pure oxygen

In method I, the arterial blood was centrifuged and the oxygen content of the separated plasma was determined. In method II, the dissolved oxygen of the blood was calculated by subtracting the oxygen bound by hemoglobin from the total oxygen content of arterial blood.

In the last three cases because of the improved technique of cooling, no correction was made for the oxygen consumption of blood cells.

OXYGEN INHALATION	O_2 PRESSURE IN THE ALVEOLAR AIR	METHOD I		METHOD II	
		O_2 pressure in the arterial blood	Δ pO_2 Art.- pO_2 Alv.	O_2 pressure in the arterial blood	Δ pO_2 Art.- pO_2 Alv.
minutes	mm. Hg	mm. Hg	mm. Hg	mm. Hg	mm. Hg
30	652	650	-2	598	-54
15	561	540	-21	656	+95
25	667	604	-63	517	-150
23	658	640	-18	556	-102
35	681	646	-35	594	-87
19	662	625	-37	498	-127
28	648	540	-108	466	-182
17	678	675	-3	463	-215
Average.....		-35.8 \pm 19.6			-126.4

lower than in the alveolar air, the average difference being 35.8 mm. Hg. With method II, the arterial O_2 pressure appeared higher than that of the alveolar air in one case, and lower in the remainder. As in this method the dissolved O_2 is found by difference, the erratic result can be explained by assuming that two abnormally high analytical errors were added. In the other 7 cases the O_2 pressure of the arterial blood obtained with method II was lower than that of method I. This result can be explained by the findings of Roughton et al. (1944), who found that the O_2 capacity of the arterial blood, as measured by the tonometer method, is about 2 per cent higher than the value in the blood at the moment of withdrawal from the artery. As this O_2 capacity has to be subtracted from the total O_2 content, a high value will give too low a figure for the dissolved O_2 in blood. In table 5, the values of the O_2 capacity obtained by the tono-

meter were compared with those calculated by subtracting from the total oxygen content of the arterial blood the oxygen dissolved in it. The latter values were calculated from the arterial oxygen pressure, obtained by method I and the Sendroy et al. formula. It can be seen that in all cases but one, the O₂ capacities obtained with the tonometer were higher than those secured with the other method.

DISCUSSION. In discussing the results, we shall assume that the oxygen exchange through the alveolar membrane follows the general laws of gas diffusion. In the eight normal subjects studied breathing pure oxygen, the arterial oxygen pressure was on the average 35.8 mm. Hg lower than that of alveolar air. According to table 2 the maximal error of method I is not likely to be over 40 mm. Hg. The standard error of the mean was ± 11 mm. Hg⁴. Therefore it is as-

TABLE 5

Comparison of the oxygen capacity of the same sample of arterial blood determined by 1, equilibrating the sample in a tonometer with a known pressure of oxygen, and 2, subtracting from the total oxygen, as found by analysis of the arterial blood, the dissolved oxygen calculated from the oxygen content of the plasma

O ₂ CAPACITY FOUND WITH TONOMETER	O ₂ CAPACITY FOUND IN THE ART. BLOOD	DIFFERENCE COLUMN 1-2	BLOOD IN TONOMETER
Vol. %	Vol. %	Vol. %	cc.
19.25	19.61	-0.36	4
18.88	18.72	+0.16	8
20.07	19.78	+0.29	6
20.46	20.18	+0.28	6
21.00	20.78	+0.22	6
19.77	19.38	+0.39	4
19.56	19.25	+0.31	8
20.48	19.83	+0.65	7
Average.....		+0.24	

sumed that the average difference is significant; the following discussion is based on the average values. The meaning of the difference between alveolar and arterial O₂ pressure deserves further analysis. According to previous work it was generally held that in the normal subject breathing air, at rest and sea level, the Δp_{O_2} (difference in O₂ tension) between alveolar air and arterial blood would amount to something greater than 20 mm. Hg⁵. However, recent workers have challenged that conclusion. Roughton et al. (1944), Comroe and Dripps (1944), Drabkin et al. (1944) using different techniques found the O₂ arterial pressure to be about 98 mm. Hg, which practically erases the Δp_{O_2} .

⁴ The formula used was $f = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$ and corrected for the smallness of the sample with the factor $\sqrt{\frac{n}{(n-1)}}$.

⁵ For several reasons this difference cannot be considered as a diffusion gradient.

If some alveolar sacs are collapsed, or their respiratory bronchioles occluded, the results will be similar to an arterio-venous shunt, as was previously discussed. If their bronchioles are patent, the O_2 content is likely to be high, even if the alveoli are poorly ventilated.

In the early stages of oxygen inhalation nitrogen is being washed out from the blood; in the poorly ventilated alveoli pN_2 will be higher than in the blood. The nitrogen will therefore diffuse into the blood, its place being taken over by the oxygen through the open bronchiole. Therefore, the saturation of blood leaving these alveoli must be complete.

As a simple calculation will show, our Δ could be explained by assuming various degrees of mixing, for instance, if 70 per cent of the alveoli had a pO_2 of 600 mm. Hg, and the remainder a pO_2 of 500 mm. Hg. In this case the blood leaving the lung would have a pO_2 of 570 mm. Hg, that is to say a ΔpO_2 of 30 mm. Hg with the higher alveolar O_2 pressure.

It is possible that the two discussed mechanisms are involved in the production of the alveolar-arterial pO_2 difference when breathing oxygen. The results of Berggren, showing that staying in bed a few days increases the ΔpO_2 , give support to the poor lung mixture hypothesis.

It would be also possible to test this by experiment. If the ΔpO_2 is due to an improper mixing in the lung, breathing oxygen during a longer period of time would diminish the difference, but no change should be observed if the difference is due to an $A - V$ shunt.

SUMMARY AND CONCLUSIONS

A method for measuring the oxygen pressure in arterial blood using the dissolved oxygen in plasma is described. A special tube for centrifugation of the blood without any gas loss has been devised.

Arterial oxygen pressure in eight normal subjects, breathing pure oxygen, was found to average 35.8 mm. Hg lower than in alveolar air.

Our results agree with Roughton et al. in showing that the hemoglobin capacity measured by the tonometer technic is too high.

Interpretation of the incomplete equilibrium between arterial blood and alveolar air is discussed.

In the normal subject the amount of blood, if any, passing from right to left heart, without being exposed to the gas phase, cannot exceed 2 per cent of the total pulmonary blood flow.

REFERENCES

- (1) BARACH, A. L. AND M. N. WOODWELL. Arch. Int. Med. **28**: 367, 1921.
- (2) BERGGREN, S. M. Acta Physiol. Scand. **4**: Suppl. XI, 1942. Cited by J. H. COMROE AND R. D. DRIPPS. This Journal **142**: 700, 1944.
- (3) COMROE, J. H. AND R. D. DRIPPS. This Journal **142**: 700, 1944.
- (4) DRABKIN, D. L., C. F. SCHMIDT, H. D. BRUNNER AND H. H. PENNES. Am. J. Med. Sci. **208**: 135, 1944.
- (5) HALDANE, J. S. AND J. G. PRIESTLEY. Respiration. Oxford, 1935.

- (6) LENNOX, W. G. AND E. L. GIBBS. J. Clin. Investigation **11**: 1155, 1932.
- (7) MATTHES, K., J. G. QUERALTÓ AND X. MALIKIOSIS. Arch. f. exper. Path. u. Pharmacol. **185**: 622, 1937.
- (8) REINHARD, E. H., C. V. MOORE, R. DUBACH AND L. J. WADE. J. Clin. Investigation **23**: 682, 1944.
- (9) ROUGHTON, F. J. W., R. C. DARLING AND W. S. ROOT. This Journal **142**: 708, 1944.
- (10) SENDROY, J., JR., R. T. DILLON AND D. D. VAN SLYKE. J. Biol. Chem. **105**: 597, 1934.
- (11) VAN SLYKE, D. D. AND J. M. NEILL. J. Biol. Chem. **61**: 523, 1924.

BIOCHEMICAL CHANGES IN THE BLOOD OF RATS AFTER REAPPLICATION OF LIMB TOURNIQUETS¹

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In the study of shock in rats produced by the application of limb tourniquets Haist and Hamilton (1) found that the animals survived after the removal of the tourniquets followed by reapplication before the animals were on the point of death. The reclamped animals rapidly recovered the ability to store glycogen in the liver when glucose was administered. The results of studies made previously in this laboratory show that when two hind limb tourniquets are applied to rats for four hours and then released, approximately 100 per cent of the animals die from shock within 24 hours (2). The results of other experiments show that when rats have been subjected to limb tourniquets for four hours followed by temporary release for one to six hours, and then replaced for 24 hours, 100 per cent of the animals survive. These findings suggest that the ischemic limb is adding to the blood stream products which in some way progressively increase the degree of shock. The indications are that the loss of fluid into the limb does not account per se for the condition and it is suggested that products resulting from the breakdown of the adenosine triphosphate energy system in the ischemic muscle, together with the early plasma loss, is responsible for the development of severe and probably irreversible shock in 7 to 8 hours after the tourniquets are released.

With these results as a basis it was decided to study the glucose, pentose, lactic and pyruvic acids, plasma inorganic phosphate and amino acid nitrogen content of the blood plasma of rats after tourniquets had been applied for four hours and then temporarily released for varying periods of time and then re-applied for different times. As a background for the blood studies it was shown previously that a typical pattern develops in the blood constituents of rats during tourniquet shock (3). The object of this study, therefore, was to determine the changes which take place in the blood constituents during the period the animals are developing shock and what changes occur when they are given an opportunity to recover by the reapplication of tourniquets. This approach to the shock problem makes it possible to ascertain which metabolic changes, as reflected by blood analyses, are critical for survival.

EXPERIMENTAL. Male rats obtained from Sprague-Dawley, Inc., and which weighed from 250 to 300 grams were divided into groups and subjected to the following procedure:

A. Tourniquets were applied for 4 hours, released for 4 hours and blood taken

¹ The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Wisconsin.

from the abdominal aorta under nembutal anesthesia at the end of this period.

B. Tourniquets were applied for 4 hours, released for 2 and then reapplied for 2 hours and the blood taken as in A.

C. Tourniquets were applied for 4 hours, released for 8 hours, and blood taken at the end of this period as in A.

D. Tourniquets were applied for 4 hours, released for 4 hours, reapplied for 4 hours and the blood taken at the end of this period as in A.

E. Tourniquets not applied, under nembutal for 4 hours and the blood taken at the end of this period.

During the first 4 hours of tourniquet treatment the animals were under nembutal anesthesia, but not at any time subsequent to this period, except for a short time when the blood samples were taken. The determinations for glucose, lactic and pyruvic acids, pentose, phosphate and amino acid nitrogen were made according to the methods described previously (3).

TABLE 1

The survival of rats after the reapplication of limb tourniquets for varying periods of time

NO. RATS	RELEASE-REAPPLICATION INTERVAL*	SURVIVAL
	<i>hrs.</i>	<i>per cent</i>
6	1	100
6	2	100
6	3	100
6	4	100
6	5	100
8	6	100
8	7	87.5

* The interval between the release of tourniquets which had been in place for 4 hours and their reapplication for 24 hours.

RESULTS AND DISCUSSION. The blood data obtained are recorded in table 2. They demonstrate that all blood constituents in the rats of group A show a definite increase above the values found in group E. When the tourniquets were released for 2 hours and then reapplied for 2 hours (group B) the rise in the values found in group A was prevented, with the exception of insignificant increases in lactic and pyruvic acids.

When the tourniquets were released for 8 hours (group C) instead of 4 hours (group A), the levels of all constituents, with the exception of pyruvic acid and glucose, rose above those observed for any of the groups, and the animals began to die after the tourniquets had been released for 6 hours (table 1). These findings indicate that progressive and abnormal changes in metabolism take place between 4 and 8 hours after removal of tourniquets. These changes, however, can be prevented by reapplication of tourniquets for the period between 4 and 8 hours after removal as shown by the blood values found in the rats of group D. Not only were further increases in the levels prevented, but decreases occurred in all the substances. The reduced values for lactic acid, phosphate

and amino acid nitrogen in the rats of group D are particularly worthy of note. When these lower values are correlated with the survival of rats with tourniquets reapplied (table 1) it seems probable that the increases in lactic acid, phosphate and amino acid nitrogen are reliable indicators of abnormal disturbances in metabolism, which result in death if allowed to continue.

SUMMARY

Determinations of blood glucose, pentose, lactic and pyruvic acids, and plasma inorganic phosphate and amino acid nitrogen were made on rats which had limb tourniquets in place for 4 hours followed by release and reapplication for different times. Correlation of these data with those obtained from survival

TABLE 2
Effect of reapplication of tourniquets on certain blood constituents of rats

TREATMENT*	GROUP NO.	GLUCOSE	PENTOSE	LACTIC ACID	PYRUVIC ACID	PLASMA PHOSPHATE	PLASMA AMINO ACID NITROGEN
		<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
T, 4+; 4-	A	150.2 (9)†	9.3 (10)	32.1 (10)	1.9 (5)	11.7 (10)	9.4 (10)
T, 4+; 2-; 2+	B	121.2 (7)	8.1 (7)	36.9 (7)	2.2 (4)	8.0 (7)	6.0 (7)
T, 4+; 8-	C	103.6 (7)	13.7 (5)	44.7 (7)	2.0 (3)	12.1 (5)	12.5 (5)
T, 4+; 4-; 4+	D	99.2 (9)	12.1 (9)	29.6 (9)	1.7 (7)	8.8 (9)	9.7 (9)
Nembutal anesthesia 4 hours	E	91.0 (7)	7.2 (7)	14.8 (7)	1.4 (7)	7.8 (7)	4.9 (7)

* T4+ = tourniquets on 4 hours; 2-, 4-, 8- = tourniquets released for these hours; 2+, 4+ = tourniquets reapplied for these hours.

† Number in parentheses is the number of rats used.

studies made on rats in which tourniquets had been reapplied after a period of release, show that there are progressive changes in the blood constituents after tourniquet release which are indicative of abnormal metabolism and approaching death. The most striking changes were found in lactic acid, phosphate and plasma amino acid nitrogen. Reapplication of tourniquets for 2 or 4 hours prevented progressive changes in the blood constituents and death of all the rats was prevented if the tourniquets were reapplied after periods of release not greater than 6 hours.

REFERENCES

- (1) HAIST, R. E. AND J. I. HAMILTON. J. Physiol. **102**: 471, 1944.
- (2) SHIPLEY, E. G., R. K. MEYER AND W. H. MCSHAN. Proc. Soc. Exper. Biol. and Med. **60**: 340, 1945.
- (3) MCSHAN, W. H., V. R. POTTER, A. GOLDMAN, E. SHIPLEY AND R. K. MEYER. This Journal **145**: 93, 1945.

EVIDENCE THAT THE MAJOR PORTION OF THE GASTRIC POTENTIAL ORIGINATES BETWEEN THE SUBMUCOSA AND MUCOSA

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In a series of experiments an attempt is being made to find out whether electrical energy furnishes the energy necessary for the production of HCl by the stomach. Studies have been reported in previous papers on the output of electrical energy by the resting stomach (4), on the correlation between the secretion of HCl and the potential difference across the stomach wall (6), and on the effect of application of a direct electric current on the rate of secretion of HCl (7).

In all of the previously reported experiments the potential difference was measured across the stomach wall, i.e., between fluid or agar in contact with the mucosal surface and fluid or agar in contact with the serosal surface. Obviously if the electromotive force giving rise to the potential difference does not originate in the secretory portion of the stomach but mainly in the muscle layers (see Hollander (3)), it would be difficult to see how this potential difference could play a direct rôle in the production of HCl. It, therefore, becomes important to determine whether the electromotive force arises in the muscle layers or in the mucosa.

In a previously published paper (5) evidence has been presented that throws some light on this problem. In these experiments the potential difference was measured across two regions of the stomach wall (two mucosal and two serosal electrodes were used). Injurious agents were applied to the mucosa at one region resulting in a very marked decrease in the potential difference across the wall at this point. The potential difference between the two mucosal sites was measured and it was found that after injury the injured region became markedly positive in the external circuit to the uninjured region. The potential difference between the serosal electrodes was also measured and showed relatively little change after injury to the mucosa. The fact that there was a large change between the mucosal electrodes and a very small change between the serosal electrodes indicates that the longitudinal resistance (resistance parallel to the surface) between the site of the origin of the electromotive force and the mucosa is much higher than the longitudinal resistance on the serosal side (in fig. 1 C of the former paper (5) $R_2 > R_4$). If the electrical resistance of the stomach is moderately uniform throughout, the conclusion would be warranted that the electromotive force originates much nearer the mucosal than the serosal surface. However, since practically nothing is known about the resistance of the various internal regions of the stomach wall, more evidence is obviously needed to

determine the site of origin of the electromotive force. In the present paper further evidence is presented demonstrating that the major portion of the potential difference across the stomach wall originates between the submucosa and mucosa. A preliminary report of these investigations appeared elsewhere (8).

METHODS. Dogs fasted for approximately 24 hours were anesthetized with sodium amytal (90 mgm. per kgm. subcutaneously). The stomach was exposed through an abdominal incision and another incision was made through the stomach wall midway between the greater and lesser curvatures. A non-polarizable electrode was introduced into the stomach through the stomach incision and placed in contact with the mucosa of the anterior wall of the stomach. Another similar electrode was placed on the serosal surface opposite to the mucosal electrode. With this arrangement fluids could be conveniently placed

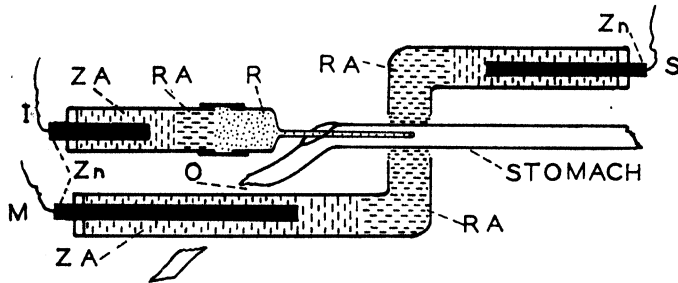


Fig. 1. Schematic drawing of the arrangement of the three electrodes referred to in the text. *S* represents the serosal electrode; *I*, the submucosal electrode; *M*, the mucosal electrode; *Zn* represents pieces of zinc metal; *ZA*, saturated zinc acetate agar; *RA*, Ringer agar; *R*, Ringer solution; *O* represents the opening in the stomach through which the mucosal electrode was introduced.

on the serosal surface. In some experiments the serosal electrode was placed on the posterior wall of the stomach and the posterior wall was thereby pushed upward through the incision exposing the mucosal surface on which the mucosal electrode was placed. With this arrangement fluids could be conveniently placed on the mucosal surface.

In other experiments a third electrode was used and this electrode was placed in the submucosa (see fig. 1). An incision about 1 cm. in length was made on the serosal side through the external muscle layers down to the submucosa. A blunt probe was inserted for a distance of several centimeters into the submucosa parallel to the surface of the stomach. The probe was withdrawn and a glass capillary containing Ringer solution and with an outside diameter of 1 mm. was inserted into the channel formed by the probe. The other end of the glass capillary was connected to a non-polarizable electrode. Mucosal and serosal electrodes were placed on the stomach opposite to the opening of the glass capillary. The distance between the edge of the serosal electrode and the incision through which the submucosal electrode was inserted was at least

10 mm. This region was wiped with cotton at frequent intervals to prevent the accumulation of fluid which would act as a shunt between the serosal and submucosal electrodes. The potential differences were measured with a Leeds and Northrup type K potentiometer and a high sensitivity galvanometer. Although the resistance of the submucosal electrode was relatively high the potential differences were easily measured to within $\frac{1}{2}$ mv.

RESULTS. *Effect of the application of alcohol and ether to the serosal surface on the potential difference across the stomach wall.* If the electromotive force arises nearer the mucosal than the serosal surface, as has been suggested above, the effect of injurious agents on the potential difference might be expected to be

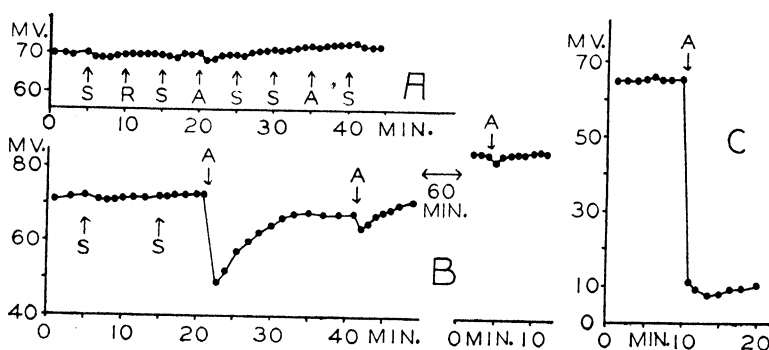


Fig. 2 A and B. Effect of 95 per cent ethyl alcohol and 0.9 per cent saline applied to the serosal surface on the potential difference measured across the stomach wall between serosal and mucosal electrodes

Fig. 2 C. Effect of three drops of 95 per cent alcohol applied to the mucosal surface on the potential difference between serosal and mucosal electrodes.

Fig. 2 A, B, C. S refers to application of 0.9 per cent saline; R, simply to removal and replacement of the serosal electrode; A, to the application of 95 per cent ethyl alcohol. The serosal electrode was positive in the external circuit to the mucosal electrode in all of these experiments. The serosal and mucosal electrodes used in these experiments are illustrated in figure 1. The submucosal electrode was not used in the experiments represented in this figure.

less marked when applied to the serosal surface than when applied to the mucosal surface. The following experiments were, therefore, designed to determine the effect on the potential difference of injurious agents applied to the serosal surface. Electrodes with a diameter of approximately 15 mm. were placed opposite each other on the mucosal and serosal surfaces. When alcohol or ether was applied to the serosal surface the serosal electrode was removed and then replaced. Control experiments were performed in which the serosal electrode was removed and replaced without the application of any fluid, and also experiments in which Ringer solution or 0.9 per cent saline was used in place of the injurious agents. It was found previously (5) that 95 per cent alcohol was more effective than ether in lowering the potential difference across the stomach wall when applied to the mucosa. Therefore, 95 per cent ethyl alcohol was used in most of the experiments in this and following sections.

Figure 2 A shows an experiment in which 95 per cent ethyl alcohol was applied to the serosal surface. It can be seen that in these experiments the application of alcohol was not followed by a significant change in the potential difference. The change in the potential difference was of the same order of magnitude as the change after saline and after simply removing and replacing the serosal electrode. Over twenty experiments in six dogs were performed in which alcohol (sixteen experiments) or ether (five experiments) was applied to the serosal surface in amounts ranging from a few drops to over 1 ml. In all but one of these experiments there was no significant change in the potential following the application of alcohol or ether. The results of this one experiment are presented in figure 2 B. It can be seen that after the first application of alcohol ($\frac{1}{2}$ ml.) there was a decrease in the potential followed by a return of the potential to over 90 per cent of its original value within 15 minutes. A second application of alcohol ($\frac{1}{2}$ ml.) was followed by a much smaller decrease of the potential. Approximately 70 minutes later a third application ($\frac{2}{3}$ ml.) was followed by no more of a change in potential than occurred in some of the experiments in which saline was applied to the serosa or in which the serosal electrode was simply removed and replaced.

In the previously reported experiments (5) in which application of alcohol to the mucosal surface resulted in a very marked decrease in the potential difference, pernoston was used as the anesthetic agent. Since amytal was used in the present experiments the possibility arises that application of alcohol to the mucosa of dogs anesthetized with amytal may not result in a fall in the potential. In order to test this possibility alcohol was applied to the surface of the mucosa in six experiments on three dogs anesthetized with amytal. In every one of these experiments there was a profound fall in the potential. The experiment illustrated in figure 2 C shows an experiment in which three drops of 95 per cent alcohol were applied to the mucosal surface. It can be seen that the application of alcohol was followed by a very marked fall in the potential difference. Application of six to ten drops of alcohol always resulted in a fall of the potential to the neighborhood of 10 millivolts or less.

It should be pointed out that in all of the above experiments the orientation of the potential difference was the same as that found in the previously reported experiments, i.e., the serosal electrode was positive in the external circuit to the mucosal electrode.

Potential differences measured with submucosal, mucosal, and serosal electrodes. In the above experiments it has been demonstrated that alcohol produces a much greater change in the potential when applied to the mucosal than when applied to the serosal surface. Assuming that alcohol penetrates the serosal surface at least as readily as it does the mucosal surface, the above evidence would indicate that the seat of the electromotive force is nearer the mucosal than the serosal surface.

Further and probably more conclusive evidence was obtained from potential difference measurements between the regular mucosal and serosal electrodes and a third electrode placed in the submucosa. Three experiments on three

different dogs were performed with this technique. Two other experiments were performed with essentially this technique except that a portion of the stomach was placed in a lucite chamber (see below).

A typical experiment (readings before the injection of histamine) is illustrated in figure 3 A. It can be seen that the potential difference in the resting stomach between the submucosal and mucosal electrodes has essentially the same magnitude as that between the serosal and mucosal electrodes. After application of the electrodes there was a tendency for the potential difference to rise and

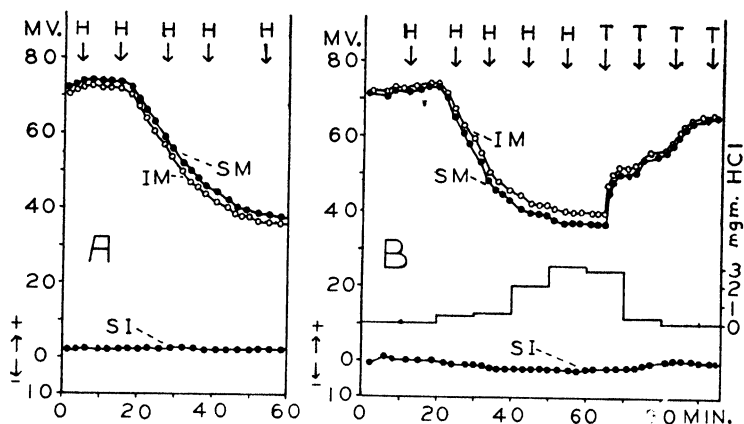


Fig. 3. A and B. The effect of histamine on the potential differences between the serosal electrode and the submucosal electrode (SI), the submucosal electrode and the mucosal electrode (IM), and the serosal and mucosal electrodes (SM). The arrows labeled *H* refer to the subcutaneous injection of histamine (1 mgm. of histamine diphosphate at each injection). The abscissa represents time in minutes. The + sign means that the electrode represented by the first letter is positive in the external circuit to the electrode represented by the second letter (and similarly for a negative sign).

Fig. 3. A. The experimental arrangement is illustrated in figure 1.

Fig. 3. B. The experimental set-up similar to figure 1 except that a portion of the stomach was placed in a lucite chamber described previously (7). In this arrangement the mucosal electrode was the same as that used in the chamber, the other two electrodes the same as shown in figure 1. The line without circles represents the rate of secretion of HCl in milligrams of HCl per ten minutes. The arrows labeled *T* represent the intravenous administration of sodium thiocyanate (1.5 grams of NaCNS at each injection).

then level off. This has been observed and reported before (5) in experiments where only mucosal and serosal electrodes were used. Table 1 shows typical values of the potential differences between the three electrodes in the five experiments after the potentials had leveled off. It can be seen from the data presented in this table that the values for the potential difference between the serosal and mucosal electrodes were essentially the same as those reported in previous papers. This finding would indicate that the presence of the submucosal electrode does not in itself significantly modify the potential difference.

The potential difference between the serosal and submucosal electrode was only a small fraction of the total potential difference across the stomach wall.

In all of the experiments, including the readings after histamine stimulation (see below), a total of approximately two hundred series of readings were made. The range of the values of the individual readings between the serosal and submucosal electrodes is given in the last column of table 1. It can be seen that the potential difference between these electrodes was never greater than 4 millivolts.

Potential differences between the three electrodes after histamine and thiocyanate. In previous experiments (6) it was shown that the potential difference across the stomach wall, following histamine stimulation, decreased from its initial resting value to the neighborhood of 40 mv. and that this decrease was associated with the onset of the secretion of HCl. It was also shown (10) that after the administration of thiocyanate the secretion of HCl was abolished and the potential returned to approximately the original resting level. The following experiments were designed to test the hypothesis that these changes in potential are not due to the establishment of a potential difference across the muscle

TABLE 1

Potential differences in millivolts between the three electrodes illustrated in figure 1. See text and description of figure 3 for an interpretation of column heads. Last column gives the maximum positive and negative values for the potential differences between the serosal and submucosal electrodes

DOG	SI	IM	SM	RANGE OF SI
1	+1.0	+73.5	+75	0 to +3.5
2	-2.5	+68	+66	-4 to +1
3	+1.0	+58	+59	-1 to +1
4	+3.5	+71.5	+75	+0.5 to +4
5	+1.0	+90.5	+91	-2 to +3

layers oriented in the opposite direction to the over-all potential difference across the stomach wall, but to changes in the potential difference between the submucosa and mucosa.

With the three electrodes in place histamine was administered subcutaneously. In figure 3 A, a typical experiment, it can be seen that the potential difference between the submucosal and mucosal electrodes decreased and that the potential difference between the serosal and submucosal electrodes did not show a significant change. The changes in the potential difference between the submucosal and mucosal electrodes always closely followed those between the serosal and mucosal electrodes.

In these experiments it is difficult to evaluate the change in the potential difference at the junction of the mucosal electrode and the mucosa during the secretion of HCl. The presence of HCl at this junction undoubtedly sets up a potential difference oriented in the opposite direction to the over-all potential of the stomach. Its magnitude would depend on the pH of the fluid at this junction. The HCl would diffuse into the agar and a potential might persist at this junction even though the stomach stopped secreting HCl (see (9)).

Therefore, it would be difficult to evaluate the changes in potential between the mucosal electrode and one of the other electrodes after inhibition of secretion following thiocyanate administration. Therefore, in the thiocyanate experiments a portion of the stomach was placed in a lucite chamber (see (7)) and a submucosal electrode was placed in this portion of the stomach. A small serosal electrode (diameter 4 mm.) was placed opposite the opening of the submucosal electrode. With this technique the potential set up by the HCl of the gastric juice does not persist for long after the stomach stops secreting HCl because the fluid in contact with the mucosa is changed every 10 minutes. Two experiments were performed with this technique. The results in both experiments were essentially the same. One of these experiments is shown in figure 3 B. The rate of secretion of HCl was also measured as described previously (7). It can be seen that there is very little change in the potential difference between the serosal and submucosal electrodes following thiocyanate administration, and that the increase in the potential difference occurs between the submucosal and mucosal electrodes.

Effect of alcohol applied to the submucosa on the potential difference between the submucosa and mucosa. The foregoing experiments demonstrate that the electromotive force (or forces) giving rise to the potential difference across the stomach wall originates somewhere between the mucosal surface and the submucosa. Application of alcohol directly to the submucosa might be expected to yield information that would enable one to localize further the site of origin of the electromotive force.

Experiments were performed in which the muscle layers were cut through exposing the submucosa. An electrode (diameter 4 mm.) was placed directly on the submucosa and another similar electrode was placed opposite on the mucosal surface. With this arrangement alcohol could be placed directly on the submucosa. It was found that with this technique the magnitude of the potential difference was essentially the same as the magnitude reported before between serosal and mucosal electrodes.

This latter finding indicates that the mucosa between the electrodes is receiving an adequate blood supply since it has been reported (1, 2) that the potential difference across the stomach is dependent on an adequate blood supply. This finding has been confirmed by the present writer. With the stomach placed in the previously described chamber (7) it was found that interruption of the blood flow resulted in a fall of the potential to approximately zero. The potential difference started falling within a matter of seconds after the blood supply was interrupted. Since the potential difference was maintained under the present conditions, it is reasonable to conclude that the mucosa between the submucosal and mucosal electrodes is receiving an adequate blood supply.

A total of eighteen experiments on three dogs were performed in which 95 per cent ethyl alcohol (fifteen experiments) or ether (three experiments) was applied directly to the submucosa in amounts ranging from a few drops to 1 ml. There was no significant change in the potential difference in any of these experiments. The results are so similar to those presented in figure 2 A in which

alcohol was applied to the serosa that it is considered unnecessary to present another figure.

DISCUSSION. In the experiments in which the submucosal electrode was used the question arises as to how large the diffusion potential would be that is set up at the boundary between the Ringer solution and the fluid in the submucosa. If this diffusion potential were relatively large then it would be theoretically possible for it to mask an equally large potential difference, oriented in the opposite direction, across the external muscle layers. A consideration of the possible composition of the submucosal fluid would lead one, on the basis of our knowledge of diffusion potentials, to expect the magnitude of this diffusion potential to be relatively insignificant. However, to test certain possibilities an experimental arrangement was set up that is illustrated by the following:

Zn	Zn Ac	Ringer	Ringer	Experimental	Sat.	Ringer	Zn Ac	Zn
	Agar	Agar	Solution	Solution	KCl	Agar	Agar	

It was found that changing the experimental solution from Ringer to a variety of solutions never resulted in the establishment of a potential difference at the Ringer-experimental solution junction of over 2 millivolts. Changing the experimental solution from Ringer to saline resulted in a change in potential difference of only a fraction of a millivolt. It is a real possibility that the submucosal fluid under the experimental conditions might contain a relatively high concentration of protein. As a means of testing the influence of a fluid with a high protein content, plasma was used as the experimental fluid. It was found that the diffusion potential set up between Ringer's and plasma was less than 2 millivolts. On the basis of these observations it is concluded that the magnitude of the potential at this junction is probably of a relatively low value.

It will be recalled that in the experiments in which alcohol or ether was applied to the serosa there was one exception to the finding that these agents produced no significant change in the potential difference across the stomach wall. In this exception it is possible that the decrease in the potential difference might be due to the establishment of a potential difference across the muscle layers in the opposite direction to the over-all potential or that it might be due to a change in the potential difference between the submucosa and mucosa. It should be pointed out in this connection that mechanical stimulation of the stomach may result in a temporary decrease in the potential difference (11). If excessive pressure is applied to the stomach via the electrodes the potential difference decreases. The magnitude of the decrease and time for recovery are related to the amount of pressure applied. It is possible that in the case of the above mentioned exception excessive pressure may have been exerted when the electrode was replaced on the serosa. There are other possible explanations of this exception, but on account of the lack of sufficient experimental data the writer does not deem it profitable to discuss them at this time.

On the basis of the other experiments reported in the present paper the conclusion is warranted that the electromotive force (or forces) giving rise to the

potential difference originates not in the external muscle layers but somewhere between the submucosa and the mucosal surface.

There is the possibility that the electromotive force might originate in the muscularis mucosae. However, since a significant fraction does not originate in the external muscle layers, it seems unlikely that it originates in the muscularis mucosae. The fact that application of alcohol directly to the submucosa does not result in a significant change in the potential difference indicates that the electromotive force (or forces) originates nearer the lumen surface of the mucosa than the submucosal surface of the mucosa. In conclusion it should be pointed out that, since the electromotive force originates in the secretory portion of the stomach, it is possible that it plays a direct rôle in the formation of the HCl of the gastric secretion.

SUMMARY

An attempt has been made to find out whether the electromotive force giving rise to the potential difference across the stomach wall originates in the muscle layers or in the secretory portion of the stomach. An analysis of previously published data in which it was found that 95 per cent ethyl alcohol or ether applied to the mucosal surface resulted in a marked decrease of the potential difference across the stomach wall indicates that the electromotive force originates near the mucosal surface. In the experiments reported in the present paper it was found that with one exception application of these same agents to the serosal surface did not result in a significant change in the potential difference. In experiments in which an electrode was placed in the submucosa (see fig. 1) it was found that the electromotive force originates between the submucosa and mucosa. It was also found that the changes in potential that occur following histamine and thiocyanate injection are due to changes in the potential difference between the submucosa and mucosa. It was also found that application of alcohol directly to the submucosa does not result in a significant change in the potential difference between electrodes on the mucosa and submucosa. It is concluded that the electromotive force originates in the secretory portion of the stomach.

REFERENCES

- (1) BOHLEN, F. *Pflüger's Arch.* **57**: 97, 1894.
- (2) BOHLEN, F. IN W. BIEDERMANN. *Electrophysiology*, p. 503. Macmillan Co., Ltd., London, 1896.
- (3) HOLLANDER, F. *Gastroenterology* **3**: 319, 1944.
- (4) REHM, W. S. *This Journal* **139**: 1, 1943.
- (5) REHM, W. S. *This Journal* **140**: 720, 1944.
- (6) REHM, W. S. *This Journal* **141**: 537, 1944.
- (7) REHM, W. S. *This Journal* **144**: 115, 1945.
- (8) REHM, W. S. *Federation Proc.* **5**: 85, 1946.
- (9) REHM, W. S. AND A. J. ENELow. *Gastroenterology* **3**: 306, 1944.
- (10) REHM, W. S. AND A. J. ENELow. *This Journal* **144**: 701, 1945.
- (11) REHM, W. S. Unpublished.

A COMPARATIVE STUDY OF THE EFFECTS OF OXYGEN LACK ON PERIPHERAL NERVE

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The majority of the observations on the effects of oxygen lack on peripheral nerve excitability and conductivity have been made using frog nerves (2, 4, 5, 8), though the asphyxiation of mammalian (9, 4) and crustacean nerves (1, 3) has also been described. When the information on nerve asphyxiation is reviewed, it is at once evident that without oxygen the nerves of mammals remain excitable a shorter time than do those of lower animals. Metabolic studies show that mammalian nerves have a higher oxygen consumption than the nerves of lower animals (4, 5). The possibility of an inverse relationship between oxygen uptake rate and the survival time during anoxia has been suggested (4). The present investigation was undertaken to test this point and to study the effects of anoxia on peripheral nerves of some widely different species.

METHODS. The metabolic rates and survival times of peripheral nerves of crustaceans, frogs, and mammals were recorded. The nerves used included:

A. Whole nerves, or large and small fiber bundles, in the meropodite of the walking leg of the spiny rock lobster (*Panulirus interruptus*).

B. Whole nerves, or large and small fiber bundles, in the meropodite of the claw of the fresh-water crayfish (*Cambarus clarkii*).

C. Sciatic nerves of the leopard frog (*Rana pipiens*) and the bullfrog (*Rana catesbiana*).

D. Peroneal and saphenous nerves of the dog.

E. Peroneal, saphenous, tibial, medial and phrenic nerves of the cat.

F. Peroneal, saphenous, tibial, medial, phrenic and radial nerves of the rabbit.

Since injury may result from simply suspending the nerves from electrode wires (10), the chamber diagrammed in figure 1 was devised for experiments with mammalian and frog nerve. It consisted of a rectangular box 2 inches long and one inch in width and height made from $\frac{1}{8}$ inch thick lucite. A ledge, A, with a shallow trough, B, was cut from a lucite block and glued to the bottom of the box. Silver-silver chloride electrodes, C, were imbedded in the ledge so that their upper surfaces were flush with the bottom of the trough. A nerve when placed in the trough was thus supported along its whole length and made good electrical contact with the wires. Gas inlets, D, and outlets, E, were inserted through holes in the side and the lid, F, of the chamber.

The nerve after removal from the animal was kept in Tyrode solution for 15 to 20 minutes and then placed in the trough. The moat, H, was partly filled with the same solution. The lid was screwed, G, on the box using vaseline to attain a gas-tight seal.

The chamber was then placed in a metal container immersed in a constant temperature bath. Mammalian nerves were asphyxiated at 38°, frog nerves at 25°. Ten to fifteen minutes were allowed for temperature equilibrium to be reached. During this time oxygen saturated with water vapor was passed through the container. Commercial nitrogen freed from oxygen by passing over heated copper gauze and saturated with water vapor was then introduced into the chamber. The gas pressure inside the box was held slightly above the atmospheric pressure as a further precaution against any leakage of air from the outside into the chamber.

The nerve was stimulated with single shocks from a thyatron square wave generator and the action potentials were recorded with a cathode ray oscillo-

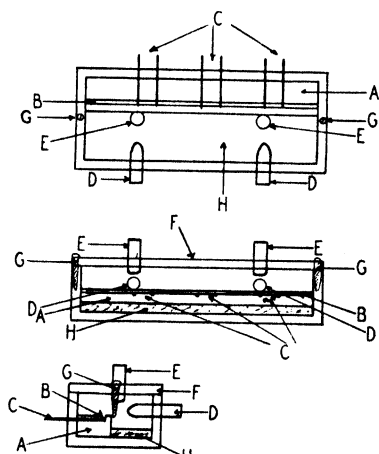


Fig. 1

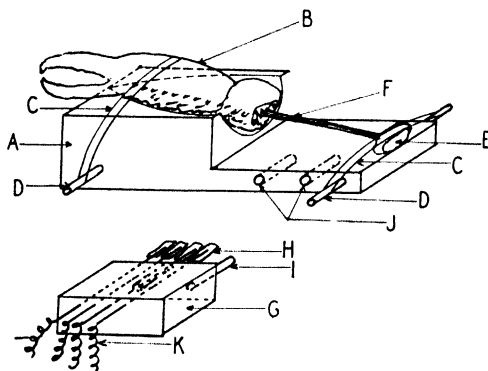


Fig. 2

Fig. 1. Diagram of Lucite Chamber for asphyxiation of mammalian and frog nerves. For explanation, see text.

Fig. 2. Diagram of Lucite nerve holder for crustacean nerves (see text for description). This holder fitted into a Lucite box not shown in figure, which served as the gas-tight asphyxiation compartment.

graph. The threshold of excitation and the spike amplitude were determined every minute. The survival time of a nerve is defined as the time from the start of exposure to nitrogen until complete extinction of the spike.

The lucite trough proved unsatisfactory for the very thin crustacean nerve fiber bundles because fluid collecting around the fibers depressed the spike amplitude and raised the threshold of stimulation. Therefore, the following method was devised. The limb was severed from the animal proximal to the ischiopodite and the shell and muscles of the meropodite were removed, leaving only the nerve connecting the ischiopodite with the carpopodite.

The base section, A, of a special holder (fig. 2) was shaped to hold the propodite, B, of either lobster or crayfish. The propodite was fastened to the limb-holder with elastic bands, C, looped over the rods, D, and the ischiopodite joint, E, was secured in similar fashion. The nerve, F, was thus suspended still

attached to its muscles, which has been found to prolong the excitability of the nerve markedly (10). The holder and limb were then immersed in van Harreveld solution (6) for crayfish, or sea water for lobster, and the nerve fiber bundles were prepared (7).

TABLE 1

The survival times of mammalian nerves asphyxiated immediately after removal from the animal

NERVE TYPE	RABBIT NERVE SURVIVAL TIME	CAT NERVE SURVIVAL TIME	DOG NERVE SURVIVAL TIME
	<i>mins.</i>	<i>mins.</i>	<i>mins.</i>
Tibial.....	18	45	
Tibial.....	16	30	
Tibial.....	30		
Tibial.....	20		
Tibial.....	28		
Tibial.....	20		
Tibial.....	16		
Peroneal.....	28	25	26
Peroneal.....	15	45	35
Peroneal.....	35	33	
Peroneal.....	28	43	
Peroneal.....	35		
Peroneal.....	23		
Peroneal.....	22		
Peroneal.....	22		
Peroneal.....	17		
Peroneal.....	20		
Peroneal.....	30		
Peroneal.....	30		
Peroneal.....	16		
Peroneal.....	16		
Saphenous.....	18	35	35
Saphenous.....	16		
Medial.....	23	20	
Medial.....	15		
Radial.....	25		
Phrenic.....	32	18	
Phrenic.....	35	35	
Average.....	24	33	33
	Standard error = 1 min.	Standard error = 3 mins.	Standard error = 3 mins.

An electrode holder was made of lucite block, *G*, into which were sealed four platinum wire electrodes to which were fastened small strips of filter paper, *H*, soaked in physiological solution or sea water. The filter paper strips were broad compared to the gaps between them, thus supporting the exposed portion of the nerve evenly along its whole length.

After the preparation of the fiber bundles was completed, the electrode holder

was fitted to the limb holder while both were immersed, by inserting the prongs, *I*, into the holes, *J*, in the base. The nerve was carefully raised and the four filter-paper-platinum-wire electrodes slipped underneath. Then the combined holders were raised from the solution, the nerve coming to rest on the filter-paper strips. The preparation was then placed in a lucite box 6 inches long, 3 inches wide and 2 inches high complete with gas inlets and outlets as described for the other chamber. This box was then put in the metal container in the

TABLE 2

The survival times of frog and lobster nerves asphyxiated immediately after removal from the animal

RANA PIPIENS	RANA CATESBIANA	PANULIRUS INTERRUPTUS
<i>mins.</i>	<i>mins.</i>	<i>mins.</i>
70	360	40
220	120	45
180	240	45
180	360	60
180	190	55
200	150	60
120	200	75*
200		60
180		90
180		90
95		120
100		60
120		105
		70
		75*
		75*
		80
		90
		95
		85
		120
Average .170	230	76
Standard error = 13.9 mins.	Standard error = 36.2 mins.	Standard error = 5 mins.

constant temperature bath. Crayfish nerves were asphyxiated at 25° and lobster nerves at 19°.

The oxygen uptake rates were measured using the Warburg method. Vessels with volumes of about 3 cc. were used. The metabolic rates were determined at the same temperatures as used in the asphyxiation experiments.

RESULTS. *Survival time.* The survival times of mammalian nerves are listed in table 1. The average survival time of 28 rabbit nerves was 24 minutes with a standard error of 1 minute, of ten cat nerves 33 minutes with a standard error of 3 minutes, and of three dog nerves 33 minutes also with a standard

error of 3 minutes. Lehmann (9) gives 25 to 35 minutes as the survival time for cat nerves, and Gerard (4) reports 30 minutes survival time for a dog peroneal. All the nerves taken from one rabbit (or cat) survived anoxia about equally long. Thus the nerve survival time is a characteristic of each individual animal. Though Lehmann (9) claims that the saphenous has a shorter survival time than other nerves examined, no evidence was found that correlation exists between specific nerves and survival times.

The survival times of nerves of *R. pipiens* and *R. catesbiana* are shown in table 2. The average survival time for *R. pipiens* is 170 minutes with a standard error of 13.9 minutes. The average survival time for *R. catesbiana* is 230 minutes with a standard error of 36.2 minutes. These results are in accord with those of previous observers mentioned above.

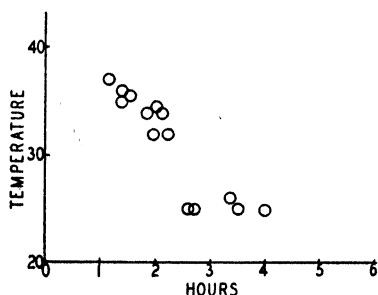


Fig. 3

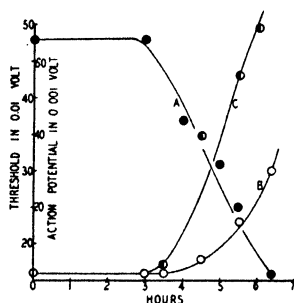


Fig. 4

Fig. 3. Effect of temperature on survival time, showing the survival times in hours of 14 frog nerves (*R. catesbiana*) at varying temperatures.

Fig. 4. The change of action potential and threshold of frog nerve during asphyxiation. The action potential amplitude (A) is recorded with solid circles and is read with the figures on the right of the ordinate (millivolts). The threshold recorded with leads 2 cm. from the cut end of the nerve (B), open circles, and with leads .5 cm. from the cut end (C), half filled circles, is read using the figures on the left of the ordinate (one hundredths of a volt). The abscissa is in hours.

The average survival time of *Panulirus interruptus* nerves (table 2) was 76 minutes with a standard error of 5 minutes. Three of the 20 nerves used were asphyxiated in toto and lasted 75 minutes (note asterisks). The other seventeen were fine bundles consisting of only a few fibers, surviving 76 minutes on the average, showing that isolation of small fiber bundles has no effect on the survival time.

Thin fiber bundles of *Cambarus clarkii* maintained normal threshold and conductivity for as long as five hours in N_2 , when the experiments were terminated. In further experiments one claw of a crayfish was placed in nitrogen, the other in oxygen for five and even nine hours. Only the muscles and part of the shell of the meropodite were removed in these preparations. The nerve activity was unchanged after these periods of anoxia. In another experiment the activity of six lobster nerves, three in oxygen and three in nitrogen, was observed along with the crayfish nerves. After $3\frac{1}{2}$ hours the crayfish nerves in

oxygen and nitrogen and the lobster nerves in oxygen were all unchanged, whereas the lobster nerves in nitrogen were completely unresponsive to stimuli.

Effect of temperature on survival time. The effect of temperature on the survival time of 14 frog nerves (*R. catesbiana*) is shown in figure 3. It is clear that the survival time of these nerves is inversely proportional to the temperature.

Changes in the action potential and threshold during asphyxiation. The action potential remained unchanged after the introduction of nitrogen into the chamber for 5 to 10 minutes when rabbit nerves were used. For cat nerve this period was 8 to 15 minutes, for *R. pipiens* 50 to 100 minutes, for *R. catesbiana* 80 to 200 minutes, and for *Panulirus* 20 to 60 minutes. The decrease of the action potential after this period is illustrated in figure 4A.

If the leads were far removed from the cut end of the nerve, the diphasicity of the action potentials is maintained until extinction (fig. 5A) whereas if the leads are placed nearer the end, the diphasic potential becomes monophasic

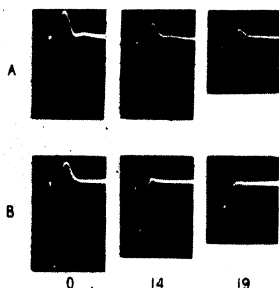


Fig. 5. A. Action potentials of cat nerve with leads about 2 cm. from cut end recorded after 0, 14 and 19 minutes after start of asphyxiation. The potential is diphasic at all times. B. Same as A with leads about .5 cm. from cut end. The potential is monophasic after 14 minutes.

during asphyxiation (fig. 5B). These observations indicate that asphyxiation affects the area around the cut end of the nerve before affecting regions some distance away.

In all species the threshold of excitation usually increased notably only some time after the decrease in the size of the action potential had started. The threshold then increased rapidly, resulting in the loss of excitability of the nerve (fig. 4B). This threshold rise was preceded in some cases by a slight decrease as has been observed by Lehman, Gerard and others. Thus the decrease in the amplitude of the action potential, measured with maximal shocks, and the threshold of excitation, investigated with just liminal shocks, do not run perfectly parallel. The first may indicate the decrease in the number of active fibers as well as a decrease in the action potential of each fiber, both of which may occur without a significant change in the excitability of the fibers remaining active. When the stimulating electrodes were placed near the cut end of the nerve, the increase in threshold began earlier (fig. 4C) and proceeded more gradually as compared to the threshold changes observed when stimulating

TABLE 3

Recovery times of irritability in mammalian and lobster nerves after asphyxiation

NERVE TYPE	BEGIN RECOVERY	COMPLETE RECOVERY
Rabbit nerves		
	<i>secs.</i>	<i>mins.</i>
Medial.....	90	8
Medial.....	60	8
Medial.....	50	8
Medial.....	60	10
Medial.....	60	5
Medial.....	60	10
Medial.....	90	10
Peroneal.....	90	10
Peroneal.....	60	
Peroneal.....	60	
Peroneal.....	60	20
Peroneal.....	60	12
Peroneal.....	120	15
Tibial.....	60	10
Tibial.....	180	10
Tibial.....	90	10
Saphenous.....	90	15
Cat nerves		
Peroneal.....	60	10
Peroneal.....	60	10
Peroneal.....	90	10
Peroneal.....	60	13
Peroneal.....	60	10
Phrenic.....	60	5
Phrenic.....	60	5
Saphenous.....	50	10
Saphenous.....	50	15
Radial.....	45	
Tibial.....	60	5
Medial.....	60	5
Dog nerves		
Peroneal.....	120	8
Peroneal.....	60	15
Tibial.....	60	10
Lobster nerves		
	<i>mins.</i>	
Whole nerve.....	5	30
Whole nerve.....	5	30
Closer bundle.....	6	30
Closer bundle.....	10	30
Closer bundle.....	10	30
Closer bundle.....	2	10

farther away from the end. This is probably due to the earlier effect of the oxygen lack near the cut end, as indicated by the changes of the action potentials. The early loss of conductivity and probably excitability at the cut end can be compensated by an increase in stimulus, which by current escape stimulates the nerve further away.

Recovery. Asphyxiated mammalian nerves recovered rapidly when exposed to oxygen or air. The data on the recovery of threshold and spike amplitude are collected in table 3. Mammalian nerves began recovery after 30 to 120 seconds in oxygen. The recovery was complete after 5 to 10 minutes. Figure 6 shows the change in threshold and spike amplitude after admission of oxygen to the chamber. Both the threshold and the spike change rapidly at first, ap-

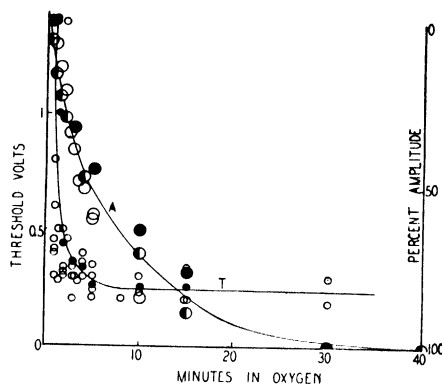


Fig. 6. The threshold and spike amplitude recovery curves of mammalian nerves in oxygen after asphyxiation. The open small circles indicate the threshold recovery of rabbit nerve. The filled-in circles indicate the recovery of cat nerve threshold. These curves are to be read using the left ordinate (in volts). The large open circles represent the spike recovery of rabbit nerve, the filled-in large circles represent recovery of spike amplitude of cat nerve, and the half-filled circles indicate spike amplitude recovery of dog nerve. These latter curves are to be read using the right ordinate in per cent original size. The abscissa (in minutes) is common for all curves.

proaching their normal values asymptotically. The recovery of the action potential is somewhat slower than the threshold recovery. The action potential reaches its normal size considerably after the threshold recovery is completed. The course of recovery of the threshold as described by Lehmann (9) was never observed.

The recovery of lobster nerves takes place more slowly than mammalian nerves, the first sign is not apparent for 5 to 20 minutes after the readmission of oxygen to the chamber (table 3). One nerve bundle recovered completely in only ten minutes, but the other five required 30 minutes. In one particular preparation, a five fiber bundle, asphyxiation was terminated when four of the fibers were inactive (fig. 7). At this time oxygen was readmitted and after five minutes one of the fibers began to recover. The conduction velocity of the fiber at first was considerably slower than that of the fiber which had remained

active, but increased rapidly so that the potentials of the two fibers were summated on the oscilloscope screen after 8 minutes. After 13 minutes another fiber began recovery also conducting slowly at first. After 20 minutes the conduction velocity had increased to equal the others. At 45 minutes a third fiber began recovery, and at 60 the fourth fiber. Both showed slow conduction velocity in the beginning.

The majority of asphyxiated frog nerves recovered rapidly. The action potential reappeared after 2 to 5 minutes and attained normal size after 15 to 20 minutes. The threshold dropped quickly and reached pre-asphyxial value in about the same time. These results are not in complete accord with all previous work (2, 8), in which it was claimed that asphyxiated frog nerve is unable to

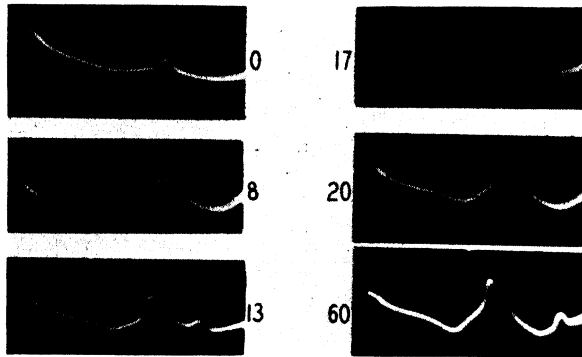


Fig. 7. Lobster nerve recovery. Recovery of action potentials by small lobster bundle of only five fibers. Asphyxiation was terminated when only one fiber was still active (upper left) and oxygen was admitted (0). After 8 minutes two fibers are active, one with a slightly slower conduction velocity causing the deflection to be broad. After 13 minutes the deflection from two fibers has narrowed and a third fiber has begun to function with definitely slow conduction placing the deflection to the right (the sweep is from left to right). After 17 minutes the velocity is greater and after 20 minutes equals those of the other two fibers. At 60 minutes the potentials of four fibers are summated in the large spike and the fifth has begun to function, but conduction is slow.

recover completely in oxygen. Gerard (4), however, reports rapid and complete recovery. Of 27 frog nerves asphyxiated in the present work, 12 recovered completely, 12 better than 50 per cent and 3 not at all. Considering the long time necessary for the asphyxiation of frog nerve the instances of poor recovery can be ascribed to failures in experimental techniques.

The effect on mammalian nerve of prolongation of asphyxiation for 1 to 2 hours after complete extinction of the excitability was also investigated. It was observed that the start of recovery could be delayed as much as 10 minutes, but the drop in threshold and rise in spike amplitude, once begun, followed the same course as seen in figure 5. Complete recovery was recorded, although in some instances the action potentials did not attain their original size. A few nerves did not recover at all, but these were usually found to be in poor condition.

The possibility of toxic metabolites collecting in high concentrations in as-

phyxiated nerves preventing recovery, as suggested by previous workers (1, 2, 8) was investigated by asphyxiating the nerves for one hour under the dryest possible conditions. This would cause the greatest concentration of these hypothetical substances, yet four rabbit nerves thus treated recovered promptly and completely.

Oxygen uptake rates. The average oxygen uptake rates of the nerves of the various species are listed in table 4. The average of 22 rabbit nerves including peroneal, saphenous and tibial is 245 cmm/gm/hr. Cat nerves, 37 tested, averaged 193 cmm/gm/hr. The average of 27 nerves from *Panulirus* was 70 cmm/gm/hr. Sixteen nerves of *R. pipiens* gave an average figure of 50 cmm/gm/hr. and fifteen bullfrog nerves averaged 29. The *Cambarus* nerves had so slight an oxygen uptake and were so small that it was necessary to include 4 or 5 in

TABLE 4

The oxygen uptake rates of nerves as recorded immediately after removal from the animal. Included are the figures of other observers in the right column

NERVE	NO. OF TESTS	TEMPERATURE	OXYGEN UPTAKE IN CCM/GM/HR	FIGURES FROM GERARD (1932)	OBSERVER
		degrees			
Rabbit.....	22	38	245 (± 11)	280	Gerard
Cat.....	37	38	193 (± 10.3)		
Dog.....				120-190	Gerard
<i>Panulirus</i>	27	19	70 (± 3.1)		
<i>Homarus</i>				80	Chang
<i>R. pipiens</i>	16	24	50† (± 3.5)	37-56*	Fenn
<i>R. catesbiana</i>	5	24	29 (± 4.4)	29	Gerard
<i>Cambarus</i>	45	24	13 (± 1.4)		

* The lowest figure was recorded in December; the highest in July. Fenn considers this as a seasonal variation. The average over the year is 41.

† This figure obtained in September.

each vessel to obtain any reading at all, and even then the error was large. The average of the 45 nerves used was 13 cmm/gm/hr. Included in the right hand column are figures taken from Gerard (5) which are in good agreement with those in the present work.

The oxygen consumption of the cut end of the nerve was investigated by comparing the oxygen uptake rate of a nerve cut in several pieces with that of a nerve left intact. Schmidt (11) has reported a higher metabolism in the region of the cut end than in the intact part. It was found that the cut nerve consumed oxygen considerably more rapidly than did whole nerve. For instance, one bullfrog sciatic cut in 8 pieces had a metabolism of 50 cmm/gm/hr. The other sciatic from the same animal left in one piece had an uptake rate of 32 cmm/gm/hr. The latter figure is normal for bullfrog nerve, but the consumption of the cut nerve was far greater than the highest figure recorded for whole nerve (35 cmm/gm/hr.).

DISCUSSION. It is immediately clear from the data that nerves with long

survival times have low oxygen uptake rates, and that nerves with short survival times have high oxygen uptake rates. Furthermore, the product of the survival time and the oxygen uptake rate proves to be a constant (about 6500 within experimental error) for all species investigated as shown in column three of table 5. Thus the survival time of nerve activity in the absence of oxygen is inversely related to the oxygen uptake rate of the nerve.

The value for *R. pipiens* is high due to the fact that the survival time, based on data taken throughout the year, has been multiplied by the oxygen uptake rate determined in September, at which time the oxygen consumption is greater than the average over the whole year (5). If the average oxygen uptake rate is used (41 cmm/gm/hr) the product is 7140, in better accord with the constant value. No value is given in the third column for *Cambarus*, since neither the survival time nor oxygen uptake rate were accurately established. The figures for the spider crab were taken from Furusawa (3) and Gerard (5).

TABLE 5

NERVE	SURVIVAL TIME	OXYGEN UPTAKE	PRODUCT
	<i>mins.</i>	<i>cmm./gm./hr.</i>	
Rabbit.....	24	245	5880
Cat.....	33	193	6369
Dog.....	33	190 (4)	6270
Spider crab.....	Approx. 50 (6)	125 (4)	6250
Panulirus.....	76	70	5320
<i>R. pipiens</i>	170	50	8500*
<i>R. catesbiana</i>	230	29	6670
<i>Cambarus</i>	>540	13	

* Using Fenn's figure from an all-year average of oxygen uptake gives 7140.

It has been shown that the survival time varies inversely with the temperature, whereas according to Gerard (5) oxygen uptake rate is directly proportional to the temperature. Thus, these temperature relationships again demonstrate the inverse proportionality between oxygen uptake rate and survival time.

Finally, it was found that the survival time is shorter and the oxygen consumption greater near the cut end of the nerve demonstrating in still another way that an inverse relationship exists between the two values.

In endeavoring to explain the ability of the nerve to function for a certain length of time without oxygen, previous workers (2, 4, 8) postulated an "oxygen reserve" existing in the nerve tissue. To satisfy the inverse relationship found here, these reserves would have to be the same in all nerves investigated. This seems remarkable, especially when the large differences in structure of the nerves used are taken into account.

The possibility that these identical reserves consist only of dissolved oxygen in the nerve tissue, though attractive, seems unlikely. A simple calculation shows that only enough oxygen can be dissolved per unit weight of rabbit nerve

to meet its requirements for about 8 minutes, far less than the 24 minutes observed survival time. A bullfrog nerve would use its dissolved oxygen in about 48 minutes and yet endures 230 minutes anoxia. Such figures are not accurate, since they do not take into account the diffusion of oxygen out of the nerve, and they are based on the questionable assumption that the metabolism of nerve remains constant throughout asphyxiation.

Schmidt (12) has demonstrated the existence of a hemin-like substance in frog nerve. Another calculation indicates that approximately 50 mgm. of hemoglobin per gram of nerve would be necessary to hold the oxygen needed to supply the tissue for the remainder of the survival time after the dissolved oxygen had been used up. Such a concentration of Hb is doubtful and hence hemin-like substances are probably not the holders of the reserve supply.

At present, no adequate explanation for the inverse relationship between survival time and oxygen consumption in peripheral nerve can be offered.

SUMMARY

A study of the effects of oxygen lack on the peripheral nerves of crustaceans, frogs and mammals is presented.

It is shown that nerves with low oxygen uptake rates have long survival times without oxygen as compared to the survival times of nerves with high oxygen uptake rates. The product of survival time and oxygen uptake rate is a constant for the species investigated (6500).

The survival time varies inversely and the oxygen uptake directly with temperature.

The survival time is shorter and the oxygen uptake rate higher near the cut end of the nerve compared to the intact part.

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REFERENCES

- (1) COWAN, S. L. *Proc. Roy. Soc.* **B115**: 216, 1934.
- (2) FILLIE, H. *Ztschr. f. allg. Physiol.* **3**: 131, 1903.
- (3) FURUSAWA, K. *J. Physiol.* **49**: 325, 1929.
- (4) GERARD, R. W. *This Journal* **92**: 498, 1930.
- (5) GERARD, R. W. *Physiol. Rev.* **12**: 469, 1932.
- (6) VAN HARREVELD, A. *Proc. Soc. Exper. Biol. and Med.* **34**: 428, 1936.
- (7) VAN HARREVELD, A. AND C. A. G. WIERSMA. *J. Physiol.* **88**: 78, 1936.
- (8) HEINBECKER, P. *This Journal* **89**: 53, 1929.
- (9) LEHMANN, J. E. *This Journal* **99**: 111, 1937.
- (10) MARMONT, G. *This Journal* **130**: 392, 1940.
- (11) SCHMIDT, F. O. *Cold Spring Harbor Symposia on Qualitative Biology* **4**: 118, 1936.
- (12) SCHMIDT, F. O. *This Journal* **95**: 650, 1930.

EXCITATION AND INHIBITION OF PHRENIC MOTONEURONES BY INFLATION OF THE LUNGS¹

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Since the classical experiments of Hering and Breuer and of Head it has been generally accepted that inspiration is inhibited by distention of the lungs. Occasional observations, however, have indicated that under certain special conditions pulmonary distention excites inspiration instead of inhibiting it. Thus Head found that after a cervical segment of each vagus nerve had been frozen and then allowed to thaw, there appeared a stage in the recovery during which artificial inflation of the lungs prolonged the inspiratory phases of respiration and shortened the expiratory phases. After more complete recovery inflation produced the usual inhibition of inspiration. Similarly Hammouda and Wilson (1935a,b) and Steffenson, Brookhart and Gesell 1937 have shown that when cervical portions of both vagus nerves are cooled to about 5°C., inflation of the lungs causes an increase instead of the normal decrease in the frequency of respiration. These observations were explained by assuming that the stimulation of inspiration is due either to a set of pulmonary receptors whose afferent fibers are less readily blocked by cold than are the fibers which inhibit inspiration, or to a lowering of blood pressure caused by the inflation.

The above observations were made with mechanical recording of the respiratory movements. If the neuromuscular activity which caused these movements is examined more closely by electrical methods, it is possible to show under more natural conditions that suitable inflation of the lungs reflexly stimulates motoneurons to inspiratory muscles. Thus Worzniak and Gesell have found that even with the vagus nerves in normal condition, properly timed "superinflation" (Gesell, 1940) of the lungs causes a brief increase in the frequency of the action potentials of various inspiratory muscles. We too have encountered this phenomenon while attempting to study inhibition of motoneurons by recording the activity of single fibers in the phrenic nerve during graded inflation of the lungs. In these experiments it was observed that inflation sometimes caused a striking increase in the frequency of discharge instead of the expected slowing. The present paper records the conditions of pulmonary inflation which lead to excitation or to inhibition of phrenic motoneurons.³ In the following paper a single fiber analysis of the afferent fibers responsible for these two reflex actions will be presented (Knowlton and Larrabee, 1946).

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METHODS. The experiments were performed on cats, usually anesthetized with Dial, or decerebrated under ether in occasional experiments. In many cases the chest wall was removed, so that volume changes of the lungs could be made without interference from the respiratory efforts of the animal. One phrenic nerve was cut and its central end freed for several centimeters from the surrounding tissues and placed in a dissecting tray. With the aid of a binocular microscope and a pair of fine steel needles the end of the nerve was then carefully split into fine strands and one of these, in which only one fiber became active during each inspiration, was selected for recording.

When it was desired to observe the influence of pulmonary inflation, the respiration pump was stopped or the tracheal cannula was occluded, and a known volume of air injected at a known rate through a side arm on the tracheal cannula.

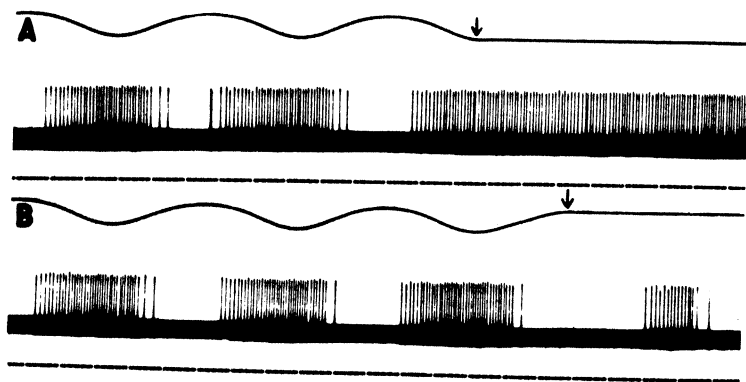


Fig. 1. Discharge from a single phrenic motoneurone during artificial ventilation with the chest wall removed. Upper line records stroke of respiration pump (inspiration upwards). At the arrow in A the pump was stopped in expiration and the inspiratory discharge was very much prolonged. At the arrow in B the pump was stopped at the height of inspiration, prolonging the expiratory pause. In this and all other records time is recorded in $\frac{1}{2}$ and $\frac{1}{10}$ sec.

For this purpose a 100 cc. syringe was driven by a motor to enable smooth and reproducible inflations. After each observation respiration was restored for a sufficient time before making another injection, to permit recovery from the slight asphyxia which presumably developed during the course of observation.

RESULTS. *Response to artificial inflation of the lungs.* A single phrenic motoneurone discharges repetitively during each inspiration (Adrian and Bronk, 1928). Under the conditions of artificial inflation employed in these experiments this discharge stops abruptly as the pump stroke inflates the lungs. When the pump is stopped in expiration the ensuing discharge lasts for several seconds (fig. 1A). When it is stopped at the height of inflation the onset of the next discharge is delayed (fig. 1B). These results are in agreement with the experiments by which Head (1889) demonstrated that distention of the lungs inhibits contraction of the diaphragm. By stopping the pump either at expiration or inspiration it is thus possible to test the effect of controlled inflations while the motoneurone is either in activity or at rest.

The two types of response with which this paper is concerned can be readily demonstrated by a series of observations in which the pump is stopped in expiration. During the prolonged motoneurone discharge which results, different amounts of air are blown into the lungs. If the lungs are held distended for a few seconds at the new volume it is found that the discharge of impulses by a phrenic motoneurone is inhibited for a length of time which is longer the more the

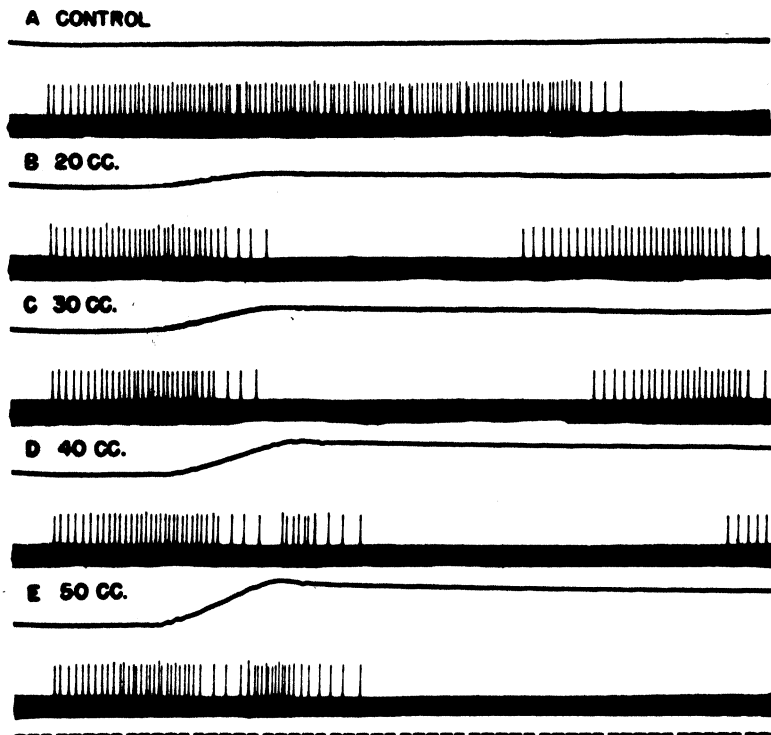


Fig. 2. Responses of a phrenic motoneurone to inflating the lungs with the volumes of air indicated on the figure. Chest wall removed. The respiration pump was stopped in expiration just before the start of each record. Inflations of 20 and 30 cc. inhibited the discharge. Larger inflations had in addition an excitant action revealed by the brief burst of impulses shortly after the start of inflation. *Upper line in this and all subsequent record indicates intratracheal pressure, increased pressure upwards.*

lungs are inflated (fig. 2). With the larger distentions; however, this period of inhibition is momentarily interrupted soon after the start of inflation by a brief burst of impulses (figs. 2D and 2E).

It is thus shown that inflation of the lungs can produce two opposite actions on the discharge of impulses by phrenic motoneurons. One action is inhibition, which is produced by all degrees of inflation and is the only apparent action of small inflations. Larger volumes have in addition an excitant action. This action is brief, for the discharge which it initiates lasts less than a second. From these observations and others to be presented later, it appears most reasonable

to assume that the inhibitory action is developed continuously throughout each period of inflation. With sufficiently large inflation, however, the excitant action can temporarily "break through" the simultaneously developed background of inhibition.

It is certain that the impulses evoked by large inflations of the lungs are discharged from the same phrenic motoneurones which are active in a spontaneous inspiration. This is shown by the fact that among many dissections we have

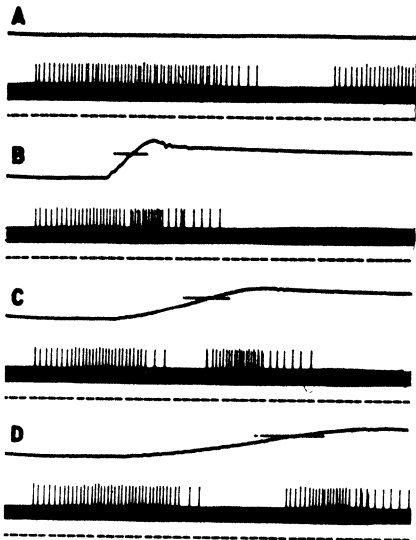


Fig. 3

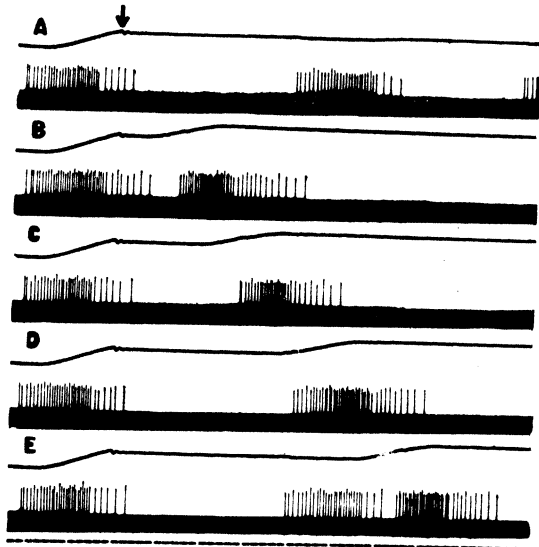


Fig. 4

Fig. 3. Responses of a phrenic motoneurone to inflating the lungs with 60 cc. of air at different rates. Chest wall removed. Pump stopped in expiration just before the start of each record. The discharge when the lungs remained deflated is shown in A, when distended at different rates in B, C and D. Horizontal lines have been drawn to intersect the intratracheal pressure tracing at the same pressure level in the last three records. A discharge of impulses is evoked when this particular level is reached at each rate of inflation.

Fig. 4. Increased discharge from a phrenic motoneurone produced by distending the lungs during various phases of respiration. Chest wall removed. Pump stopped near height of inspiration shortly after start of each record (at time indicated by arrow in A). Record A shows the periodic discharge when no further inflation was made. In the remaining records an additional 20 cc. of air was forced into the lungs at the times indicated by the rise in intratracheal pressure.

never obtained a single fiber preparation which was spontaneously active during inspiration but could not be stimulated to additional activity by sufficient inflation. Moreover the height of the action potentials was always the same in the evoked and spontaneous discharges.

The amount of distention necessary to produce the excitant action is independent of the rate of inflation within the limits investigated. This is illustrated in figure 3. When the pump was stopped in expiration the ensuing spontaneous discharge of the motoneurone continued for over two seconds (A). When 60 cc.

of air were rapidly injected (B) the excitant action was revealed by the initial acceleration of the frequency of discharge, which was followed by a slowing and finally a complete cessation of activity. In the succeeding records (C and D) the lungs were inflated more slowly, and the excitant action was correspondingly delayed. The short horizontal lines which intersect the intratracheal pressure record are all drawn at the same height above zero pressure. At each rate of inflation the evoked discharge starts when this particular level is reached.

The slow inflations in C and D of figure 3, provide additional illustrations of the fact that inhibition results from lesser volume increases than does excitation, for as the distention starts the first effect is a cessation of the discharge. This is superseded by stimulation of activity only when a critical degree of inflation has been reached.

The inspiratory excitant action of lung inflation can be effective in any phase of respiration. In the experiment reproduced in figure 4 the pump was stopped with the lungs inflated, and the motoneurone discharged groups of impulses as in normal respiration (A). Further distention of the lungs then evoked additional impulses whether the added inflation occurred early in the first expiratory pause (B), late in expiration (C), during inspiration (D), or early in the second expiratory pause (E). The excitant action in D is most clearly seen by comparison with A or E, which show that the spontaneous burst alone contains fewer impulses than the overlapping spontaneous and evoked bursts in D.

Afferent mechanism. The striking contrast between the thresholds and durations of the two opposing reflexes elicited by distention of the lungs appears to be due to differences between the thresholds and rates of adaptation of the pulmonary stretch receptors which are responsible for them. The afferent fibers for both reflexes ascend the vagus nerves, for we have confirmed Worzniak and Gesell's observation that the excitation of inspiration, like the inhibition of inspiration, is eliminated by cutting both vagi. We have, moreover, directly demonstrated the presence in the vagus of afferent fibers from two kinds of receptors stimulated by inflation of the lungs (Knowlton and Larrabee). This was done by recording the activity of individual afferent fibers. It was found that there are two sets of receptors which have thresholds and rates of adaptation to inflation which are different and which correspond closely to the thresholds and durations of the two inflation reflexes described above. From those observations it was concluded that impulses from slowly adapting receptors inhibit inspiration and that impulses from rapidly adapting receptors excite inspiration.

The above interpretation of the afferent mechanism also explains the sequence of events during slow deep inflation (fig. 3C and D) where the inspiratory motoneurone is first inhibited, then excited to activity, and finally inhibited again. Impulses which tend to inhibit inspiration presumably reach the respiratory centers in a progressively increasing number throughout the whole course of such inflations. When the relatively high threshold of the rapidly adapting receptors is reached, impulses which excite inspiration are for a brief time discharged in sufficient number to overcome the inhibition being developed by impulses concurrently arriving from the slowly adapting receptors. This interpretation, based on the

properties of two different sets of afferent fibers, contrasts with that of Gesell (1940) who suggested on the basis of the less complete knowledge of pulmonary receptors then available that one kind of afferent fiber might exert two different central actions.

The receptors responsible for both inhibition and excitation of inspiration are presumably located in the lungs. This assumption is supported by the fact that both responses to inflation may be obtained after extensive removal of the chest wall, as was done in many of our experiments. Inasmuch as both can be elicited by forcing air through a glass tube pushed down the trachea as far as the bifurcation, the sensory endings must be located below that point.

It was at first thought that the excitation of inspiration by lung distention might be secondary to changes in the circulation. The excitation is produced only by relatively large inflations, which may be expected to compress the pulmonary blood vessels. Such compression could cause a rise of venous pressure and a fall of arterial pressure, and there is some evidence that either of these

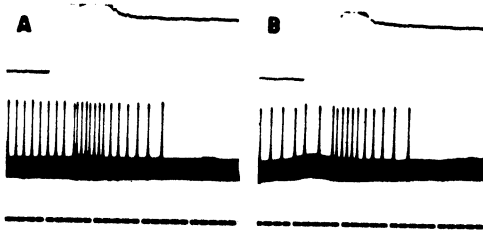


Fig. 5. Increased frequency of discharge from a single phrenic motoneurone in response to inflating the lungs with 80 cc. of air. The pulmonary artery was occluded during A and open during B. Chest wall removed.

pressure changes can reflexly stimulate respiration (Schmidt, 1932; Bainbridge, 1915). However, the short latency of the inspiratory discharge after inflation, frequently being much less than one cardiac cycle, makes it difficult to explain the discharge as a response to systemic pressure changes. And in one experiment sudden occlusion of the pulmonary artery by means of a ligature was found to have no immediate excitant action on a phrenic motoneurone. In this experiment it was also found that inflation of the lungs elicited at least as vigorous a discharge shortly after the ligature was tightened as did the same volume of inflation when the artery was not occluded (fig. 5). This indicates that the response of the pulmonary receptors is not significantly modified by changes in pulmonary blood pressure, for this pressure must have dropped profoundly as a result of the occlusion. The completeness of the occlusion was attested by the disappearance of the carotid pulse, which was also recorded although not reproduced in figure 5. From these several observations we are convinced that the excitation of inspiration is mediated not through the cardiovascular system, but rather through sensory endings directly responsive to changes in lung volume.

Rôle in normal respiration. Experiments described above distinguish the two respiratory reflexes evoked by expansion of the lungs. The way in which the re-

flex inhibition of inspiration serves to limit the depth and increase the frequency of respiration has been described by Adrian (1933). It remains to consider the rôle of the excitation of inspiration which also results from lung distention.

It has already been emphasized that the volume threshold for excitation of inspiration considerably exceeds the threshold for inhibition. Actually the extra discharge is evoked only by inflations in excess of the maximum volume reached by the lungs in normal quiet breathing. This was initially suggested to us by the observations of figure 2, for the extra burst of impulses is elicited only when the volume increase exceeds 30 cc., whereas normal tidal air is between 20 and 30 cc. in cats of the sizes used. Similar results were found in other experiments. In these experiments, however, the chest wall had been removed and it is possible that the inflations started from a level considerably different from the normal expiratory volume. Therefore we have also determined the threshold for excitation of inspiration in several animals breathing naturally with chest wall intact. By recording the intratracheal pressure as an index of respiratory activity, it was found in three anesthetized animals that an inspiratory effort was evoked only by inflations exceeding normal inspiration by more than 50 cc. In one experiment on a decerebrate preparation records were taken from a single fiber of the upper root of the phrenic nerve. In this case it was found that artificial inflations which exceeded normal inspiration by 50 to 70 cc. were required to evoke a burst of impulses.

It has thus been found that relatively large artificial inflations are required to initiate a discharge from a motoneurone which is at rest. It must be considered, however, that lesser distentions of the lungs in natural respiration might cause an afferent discharge which would be too weak by itself to *initiate* motoneurone activity, but would, nevertheless, be adequate to *accelerate* the frequency of the discharge already in progress. It is known that during each inspiration the frequency of the motor impulses to the diaphragm progressively increases (Adrian and Bronk, 1928; Gesell, Atkinson and Brown, 1940), and Gesell (1940) has suggested that this acceleration may be caused in part by afferent impulses ascending the vagus from stretch receptors in the lungs.

In order to investigate this possible rôle of inspiration-exciting impulses in normal respiration, we inserted through the thoracic wall four cannulae which were closed off by a system of rubber tubing. The tubing could be quickly opened to the atmosphere to produce a pneumothorax whenever desired, and the original conditions could readily be restored by sucking air out of the thorax before reclosing the system. When opened the total cross section of the cannula system was found by experiment to be sufficient to reduce the amount of air drawn into the lungs on inspiration by more than 75 per cent. This is adequate to reduce markedly the discharge from pulmonary stretch receptors. Therefore, if the progressive speeding of the motor discharge during inspiration is normally due in part to these afferent impulses, the frequency should rise much less rapidly during inspiration with the cannulae open.

The results of an experiment are shown in figure 6. Before the start of the record the animal had been breathing normally, and the figure shows the impulses

discharged by a motoneurone during two normal inspirations. Pneumothorax was made at the time indicated by the arrow. The subsequent motor discharge was very much prolonged. For the first 0.7 sec., however, the frequency rose exactly as in a normal inspiration. The remarkably close agreement between the frequency of discharge following pneumothorax and the major part of a normal inspiration is more clearly shown by the graph of figure 7 from another run in the same experiment. At no time did the normal discharge attain a higher rate. In fact, until the last two or three impulses in normal inspiration, the frequency was

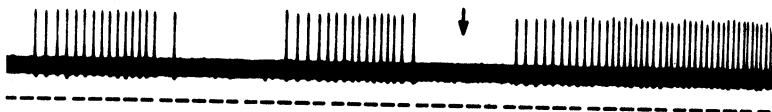


Fig. 6. Effect of pneumothorax on discharge from a phrenic motoneurone in an animal breathing naturally. Chest wall intact except for the insertion of four cannulae which were closed at the start of the record. After two normal inspirations the cannulae were suddenly opened at the arrow so that the next inspiratory effort distended the lungs only slightly. As a consequence the next discharge was very much prolonged.

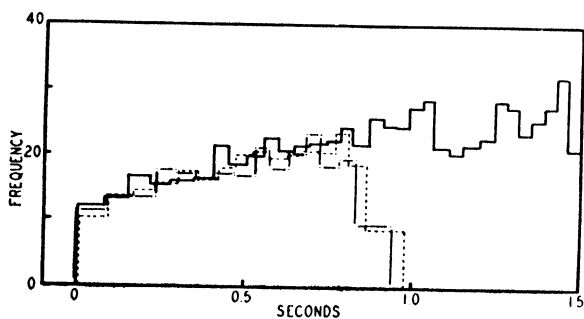


Fig. 7. Plot of the frequency of discharge from a phrenic motoneurone in a sudden-pneumothorax experiment similar to that of figure 6. The broken lines show the frequency during the last two normal inspirations before the pneumothorax and the solid line shows the frequency of the first discharge during the pneumothorax. The ordinate of each horizontal segment represents the frequency calculated from the interval between two successive impulses. The plot shows that the first 0.7 sec. of discharge is the same whether the lungs become distended or not.

quite unmodified in any way by afferent impulses initiated by the expansion of the lungs.

This observation is in agreement with the findings of Hammouda and Wilson (1932) on the dog and Head on the rabbit that the velocity of inspiration is unchanged immediately after sectioning or blocking the vagus nerves. A greater depth is reached only because the duration of inspiration is prolonged. Gesell and Moyer on the other hand concluded that inspiration was accelerated by a pulmonary-vagal reflex in the dog since in their experiments cold block of the vagus nerve reduced the speed of inspiration. But their observations were conducted with pulmonary pressure and volume artificially elevated by weighting the bell of a spirometer. The abnormally large lung volume thus produced may well have

brought into action the inspiration exciting reflex which we have shown to be inactive in the normal volume range, or caused other modifications of the respiratory pattern. Hence their findings may not be directly applicable to more natural conditions.

We, therefore, conclude that during eupneic respiration in the cat the frequency of discharge from phrenic motoneurons is not reflexly increased by the inspiration-exciting reflex described above, the reason probably being that not enough of the afferent fibers responsible for this reflex are stimulated under these conditions. This is in agreement with our studies presented elsewhere concerning afferent fibers from the lungs. If anything, a reflex slowing of the discharge would be expected, for there is abundant evidence that many of the afferent fibers which inhibit inspiration are in action even at small lung volumes (Adrian; Knowlton and Larrabee). It appears, however, that the activity of phrenic motoneurons is normally uninfluenced by *any* afferent impulses initiated by expansion of the lungs during the major part of the inspiratory cycle. This fact is worthy of careful consideration in any theory concerning the organization of the respiratory center.

The influence of pulmonary receptors on eupneic respiration may thus be summarized by saying that the inhibitory reflex cuts inspiration short. The excitation to inspiration produced by superinflation of the lungs seems to play no part in normal quiet breathing. A possible important rôle of this latter reflex in bolstering the depth of extraordinarily deep inspirations will be discussed at the end of the following paper.

SUMMARY

The influence of changes in lung volume on the discharge of impulses by single phrenic motoneurons was investigated in cats anesthetized with Dial ~~or~~ decerebrated under ether.

Inflation of the lungs inhibits the phrenic discharge for a length of time that increases with the volume of inflation. Large inflations, in addition to this well-known inhibition of inspiration, have an excitant action which is revealed by a brief burst of motor impulses as the lungs expand. This excitant action is of short duration and has a threshold which is relatively independent of the rate of distention.

The inspiration-exciting reflex is independent of vascular pressure receptors since it is not reduced during occlusion of the pulmonary artery. Both the inhibition and excitation of inspiration by lung inflation are eliminated by cutting both vagus nerves. It is suggested that these reflexes are due to impulses from two distinct sets of pulmonary receptors of different thresholds and different rates of adaptation.

Increased activity of phrenic motoneurons is caused by artificial distention of the lungs only when the lungs are inflated to a volume which exceeds that reached in eupneic respiration. Following a suddenly-induced pneumothorax the next inspiration is very much prolonged, but the frequency of discharge follows exactly the same time course as in normal inspiration up to the time when the normal dis-

charge is abruptly curtailed. Inspiration is thus uninfluenced by impulses from pulmonary stretch receptors until its termination is suddenly brought about by the inspiration-inhibiting reflex. The inspiration-exciting reflex initiated by superinflation of the lungs is inactive in normal eupneic breathing, but may serve to increase the depth of any unusually deep inspiration.

REFERENCES

- ADRIAN, E. D. *J. Physiol.* **79**: 332, 1933.
 ADRIAN, E. D. AND D. W. BRONK. *J. Physiol.* **66**: 81, 1928.
 BAINBRIDGE, F. A. *J. Physiol.* **50**: 65, 1915.
 GESELL, R. *Ergebn. Physiol.* **43**: 477, 1940.
 GESELL, R., A. R. ATKINSON AND R. C. BROWN. *This Journal* **128**: 629, 1940; **131**: 668, 1941.
 GESELL, R. AND C. MOYER. *This Journal* **131**: 674, 1941.
 HAMMOUDA, M. AND W. H. WILSON. *J. Physiol.* **74**: 81, 1932. *J. Physiol.* **83**: 292, 1935a.
 J. Physiol. **85**: 62, 1935b.
 HEAD, H. *J. Physiol.* **10**: 1, 1889.
 HERING, E. AND J. BREUER. *S. B. Akad. Wiss. Wien.* **57** (2): 672; **58** (2): 909, 1868.
 KNOWLTON, G. C. AND M. G. LARRABEE. *This Journal* **147**: 100, 1946.
 SCHMIDT, C. F. *This Journal* **102**: 94, 1932.
 STEFFENSEN, E. H., J. H. BROOKHART AND R. GESSELL. *This Journal* **119**: 517, 1937.
 WORZNIAK, J. J. AND R. GESELL. *This Journal* **126**: P658, 1939.

A UNITARY ANALYSIS OF PULMONARY VOLUME RECEPTORS¹

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It has long been recognized that changes in lung volume cause reflex alterations of respiratory activity and that the afferent pathway of these reflexes is over the vagus nerves. Adrian (1933) analyzed the afferent mechanisms involved by recording the response of single vagus nerve fibers. He concluded that there are two kinds of receptors in the lungs, one stimulated by inflation, the other by forced deflation. These receptors appeared adequate to account for the reflexes of inhibition of inspiration on inflation and the excitation of inspiration on forced deflation, which were known at that time. More recent investigations (Worzniak and Gesell, 1939) have shown, however, that activity of inspiratory motoneurons can be excited as well as inhibited by suitable inflation of the lungs. In the preceding paper we have analyzed the interrelation of these two responses to inflation (Larrabee and Knowlton, 1946). It is possible to explain the afferent mechanisms by assuming that impulses from a single inflation receptor exert two alternative central effects, exciting inspiration under some conditions, exciting expiration under others (Gesell, 1940). However, another and perhaps simpler explanation is possible if there exist not one, but two, kinds of receptors responsive to inflation. Then it can be assumed that each has only one kind of central action.

We have, therefore, re-examined, by the single fiber technique, the afferent vagal discharge produced by changes in lung volume.³ The results to be presented indicate that the receptors which respond to inflation can be divided into two well defined groups on the basis of their rates of adaptation. Certain of these receptors respond to forced deflation of the lungs, but we have been unable to find any which are not also stimulated by inflation. Our findings, therefore, differ in part from those of Adrian, and provide a more complete explanation of the recent additions to our knowledge of pulmonary reflexes.

METHODS. The experiments were performed on cats anesthetized with Dial. Usually the thorax was widely opened by removing the first five ribs and adjoining parts of the sternum so that changes in lung volume could be effected without modification by the respiratory reflexes evoked, the animal being maintained on artificial ventilation in a humidified and heated enclosure. Changes in lung volume were made by stopping the respiration pump and injecting or withdrawing a known volume of air by means of a motor-driven piston. In a few ex-

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periments the animals were allowed to breathe naturally with the chest wall intact, and the outlet of the tracheal cannula was occluded at the end of expiration just before the artificial volume change. Several centimeters of one vagus nerve were dissected free from the surrounding tissues in the neck and transected just below the ganglion nodosum. The end of the peripheral portion of the nerve was

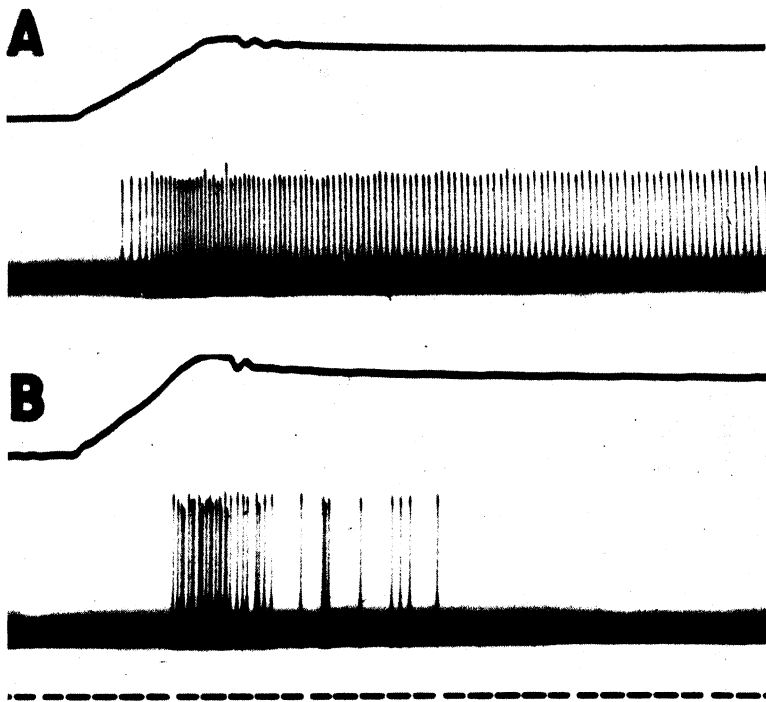


Fig. 1. Responses of two kinds of afferent fibers in the vagus to inflation of the lungs. The first receptor adapts slowly, the second very rapidly to maintained inflation of the lungs. Chest wall removed. Respiration pump stopped in expiration just before start of each record. In these and all subsequent electrical records the upper line represents intra-tracheal pressure (increased pressure upwards) and the lower line marks time in $\frac{1}{2}$ and $\frac{1}{10}$ seconds.

then placed in a small dissecting tray on which were mounted electrodes connected to the input of a vacuum tube amplifier and cathode ray oscillograph. Fine strands were successively split away from the end of the nerve trunk until a strand was prepared which contained only one active fiber, as indicated by regularity of height, form, and rhythm of the action potentials. The responses to a series of graded inflations and deflations were then recorded. In this way observations could be made on about ten different fibers during the course of each experiment.

RESULTS. *Responses to inflation.* In figure 1 are shown the responses of two

different fibers in the vagus nerve during artificial inflation of the lungs. The response of the first fiber consists of a prolonged train of impulses, with only a moderate decline in frequency over an interval of two seconds. This is similar to the activity of the vagal afferent fibers described by Adrian (1933) and by Partridge (1933). The impulses in the second fiber, however, in marked contrast to the first, completely cease at the end of one second, even though the lungs are kept distended. This rapid adaptation is characteristic of the activity in a significant number of vagal afferent fibers, for it appeared in more than one-third of the ninety fibers which we succeeded in isolating.

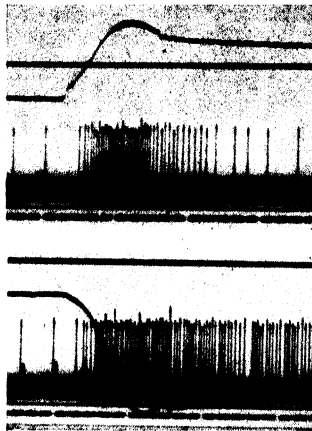


Fig. 2

Fig. 2. Fiber from a rapidly adapting receptor, showing much faster adaptation on inflation (upper) than on deflation (lower). During the control period preceding the volume change a single impulse is discharged at the same phase of each cardiac cycle. (The cardiac pulse showed faintly on the original intratracheal pressure record.) Chest wall removed.

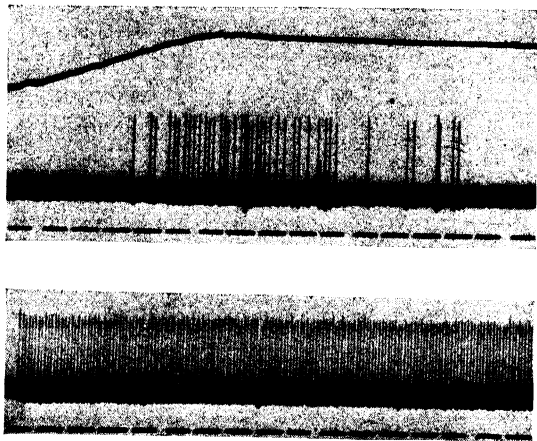


Fig. 3

Fig. 3. Response of a fiber from a rapidly adapting receptor to inflating the lungs (upper record) and to repetitive electrical stimulation of the fiber itself (lower record). Chest wall removed.

No such rapidly adapting discharges were reported by the earlier investigators, who may not have observed them, or may have assumed that the failure to continue in action was due to block of conduction at a region of the fiber injured by dissection rather than being due to a characteristic of the end-organ from which the impulses originated. Three lines of evidence convince us that injury is not responsible for the rapid decline in frequency in any appreciable number of our observations: 1. Many of the fibers which show rapid adaptation respond not only to inflation but also to forced deflation of the lungs and adapt much more slowly on deflation than on inflation (fig. 2). Injury to the fiber should impair equally the conduction of impulses initiated in either way. 2. Several fibers whose frequency of discharge declined rapidly during maintained inflation of the lungs have been stimulated directly through electrodes applied to the vagus nerve between the lungs and the recording electrodes. It was invariably found

that the fiber would conduct impulses set up by the electrical stimulation for several seconds at a frequency exceeding that reached during inflation of the lungs (fig. 3). In most of these experiments the fiber which responded to the electrical stimulation was unequivocally identified as the same one which responded to lung inflation, for the response to inflation was blocked when rapidly repeated, slightly suprathreshold electrical stimuli were being simultaneously applied, but was entirely unmodified when the electrical stimuli were just below threshold for the fiber. 3. A less direct control was obtained from the records of every fiber. It was found that the discharge usually adapted at least as rapidly during a low frequency response to weak stretch as it did during a higher frequency response to stronger stretch. This is significant because it contrasts with the failure of fibers when depressed by narcotics (Tsai, 1931) or when stimulated at very high frequency (Cattell and Grundfest, 1935; Gasser, 1937) or when believed to be injured during dissection. Under all these conditions the response fails much more rapidly at a high than at a low frequency.

We, therefore, conclude that the rapid decrement in the discharge shown in figure 1B is due to a rapid adaptation to change in lung volume by the receptor from which the impulses are discharged. Other receptors adapt much more slowly as in figure 1A. The type of adaptation is a fixed characteristic of each receptor, independent of the volume to which the lungs are distended. For example, a receptor which adapts rapidly to inflating the lungs only slightly above its threshold will also adapt rapidly to greater degrees of inflation.

The next question to be considered is whether there are two functionally distinct kinds of receptors, one which adapts rapidly and the other which adapts slowly. This can not be decided without further analysis, since we have observed many fibers in which the rates of adaptation lie between those shown in figure 1. This suggests that the examples shown may merely represent the extreme variations in one homogeneous set of end organs whose adaptation rates vary widely. To investigate this question a quantitative measure of the degree of adaptation is required. For such a measure we have calculated the percentage decline in frequency of discharge over a fixed interval of time during a maintained inflation, according to the following formula:

Adaptation index =

$$\frac{\text{Peak frequency} - \text{Average frequency during second second of inflation}}{\text{Peak frequency}}$$

× 100 per cent

This arbitrary index is 0 per cent for a receptor which does not adapt at all and 100 per cent for one which drops completely out of action by the end of the first second. The values were found to be relatively independent of the volume of inflation provided stimulation was well above threshold. For the following analysis the values obtained from inflation with at least two different volumes were averaged for each receptor.

Figure 4 shows the adaptation indices of the ninety receptors on which ade-

quate data were obtained. The large majority of values fall into two clearly defined groups: slow adaptors whose average frequency during the second second has declined by less than 55 per cent from the peak frequency, and rapid adaptors whose average frequency over the same period has declined by more than 80 per cent. These two groups are also distinguished by qualitative differences which are apparent in the original records. The discharge from the slowly adapting endings is characterized by a smooth and regular decline in frequency (fig. 1A) while the discharge from the rapid adaptors typically has a much less regular rhythm (fig. 1B, 2A, 3A, etc.). Another feature of the pattern of activity of rapidly adapting receptors is the common occurrence of a cardiac rhythm, where a single impulse or a closely spaced group of two or more impulses is discharged

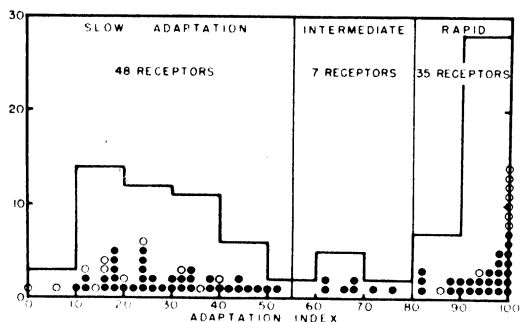


Fig. 4

Fig. 4. Distribution of adaptation indices of 90 receptors. (Adaptation index defined in text.) Each point represents one receptor, solid circles indicating observations with chest wall removed, open circles observations with chest intact. Histogram indicates number of fibers in each decade of adaptation index.

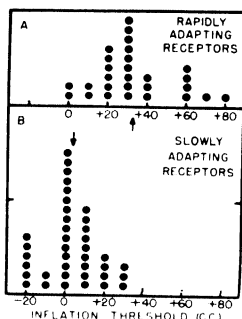


Fig. 5

Fig. 5. Thresholds of rapidly adapting endings (A) and slowly adapting endings (B) to inflation of the lungs. The abscissa of each point represents the threshold of one fiber to the nearest 10 cc. Arrows indicate average thresholds of the fibers in each group. All data obtained with chest wall removed.

during each cardiac cycle (start of fig. 2, end of fig. 6B). Under these conditions the end organ is probably stimulated primarily by the periodic distortion caused by pulsation of a nearby blood vessel rather than by the constant stretch imposed on it by lung inflation. The discharge from a slowly adapting receptor is also sometimes affected by the pulse (Adrian, 1933), but in this case the pulse effect consists of a smooth modulation of the frequency of discharge which is quite different from the single or closely grouped impulses which are discharged from a rapidly adapting ending.

In the distribution curve of figure 4, seven receptors were tentatively assigned to an intermediate group. An examination of the original records for the characteristic patterns of discharge described in the preceding paragraph shows that each of these seven end-organs clearly belongs in one or the other of the two major groups. In figure 6A for example is shown the discharge from one of the intermediate endings which resembles the discharge from slowly adapting endings be-

cause of the smooth decline in its frequency of discharge. The receptor of figure 6B, on the other hand, actually adapts very rapidly to lung inflation because after 0.4 second of activity the discharge consists only of scattered impulses which in the latter part of the record clearly recur with the cardiac cycle. These impulses are therefore presumably initiated by pulsation of the pulmonary vessels, rather than by the maintained inflation of the lungs. It is also worth noting that none of the receptors studied with chest wall intact (represented by open circles in fig. 4) fell into the intermediate classification. Thus it may be concluded that *all* of the vagal endings in the lungs whose discharges have been recorded can be divided into two distinct groups on the basis of their adaptation to maintained inflation.

These two groups of receptors are further distinguished by a difference in their thresholds to inflation of the lungs. The threshold for each fiber was determined within 10 cc. from curves showing the frequency of impulses discharged during

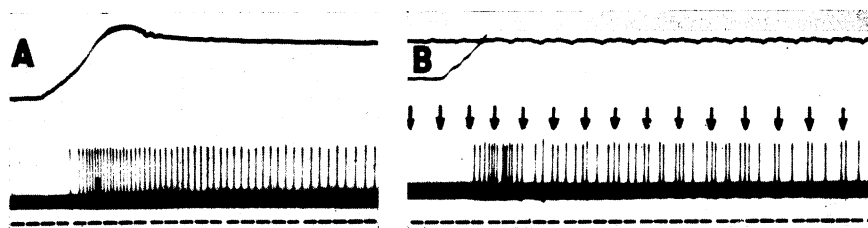


Fig. 6. Two fibers from the intermediate adaptation group of figure 4. Records from different animals with chest wall removed. The frequency of discharge in A declines smoothly, as in more slowly adapting receptors, whereas the frequency in B becomes modulated by the cardiac pulse and is irregular like that of more rapidly adapting receptors. The arrows in B are all drawn at the same phase of the carotid pulse, which is recorded at the top of the record.

the first half second after various changes in lung volume (fig. 7). Certain receptors remained in action at all volumes throughout the range investigated, and their inflation thresholds were recorded as the volume at which the impulse frequency began to increase as lung volume increased (fig. 7A, curves 2 and 4). Figures 5A and 5B show the distribution of inflation thresholds in the two adaptation groups, for fibers observed with the chest wall removed. There is a significant difference in mean thresholds, that for the rapidly adapting receptors being higher by 30 cc. It is also apparent that an inflation of 15 cc., for example, stimulates most of the slowly adapting endings without bringing into action many of the rapid adaptors.

The average threshold of the few rapidly adapting receptors studied with the chest wall intact was over 60 cc., thus being even higher than the 32 cc. indicated in figure 5A from observations with the chest wall removed. These figures are significant because they exceed the normal tidal air during eupneic breathing, which was found to be between 20 and 30 cc. in cats of the sizes used in these experiments. Thus few of these receptors can become active during quiet res-

piration, although many of them are caused to discharge vigorously under appropriate conditions, as will be described later.

Conduction velocity. Functionally different nerve fibers frequently differ in conduction velocity. Therefore, the speed of conduction of a number of fibers in each adaptation group was determined by stimulating through electrodes placed on the vagus nerve between the lungs and the recording electrodes. The velocity was calculated from the latency of the response of a single fiber measured on the face of the cathode ray tube and the conduction distance measured along the nerve. The values for 4 rapidly adapting and 10 slowly adapting fibers ranged from 8 to 35 m.p.s. The conduction velocities of all the fibers from rapidly adapting receptors were less than 15 m.p.s. (average 9.1 m.p.s.), while fibers from 7 of the 10 slow adaptors conducted faster than 15 m.p.s. (average 20.2 m.p.s. for all 10). This suggests a difference between the conduction velocities

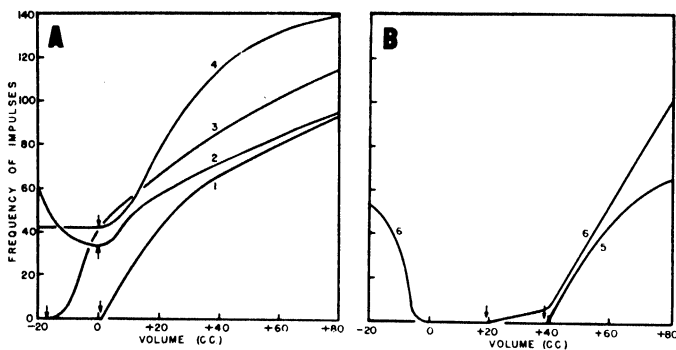


Fig. 7. Responses of typical receptors to changes in lung volume. A. Slowly adapting endings. B. Rapidly adapting endings. Ordinates are average frequencies during the first half-second after the volume changes indicated on abscissas. Arrows indicate the volumes taken as inflation thresholds for construction of figure 5.

of fibers from the two kinds of receptors, but not enough data were obtained to be sure that the difference was real.

The exact temperature of the nerve was not determined during the above experiments, but it may be assumed to have been somewhere between the temperature of the humidified operating chamber and that of the body. Accordingly it can be estimated that at body temperature the fastest fibers must have conducted at 35 to 44 m.p.s. and the slowest at 8 to 10 m.p.s. The most recent investigation of the relation between conduction velocity and fiber diameter (Gasser and Grundfest, 1939) indicates that fibers which conduct at these velocities have outside diameters which range from 8 microns to less than 2 microns. This is in substantial agreement with the estimate (Heinbecker and O'Leary, 1933) that respiratory responses are obtained by electrical stimulation of vagus nerve fibers ranging from 10 microns to 4 or less.

Response to deflation. Many of the pulmonary receptors which respond to inflation are also stimulated by forced deflation of the lungs. In experiments with

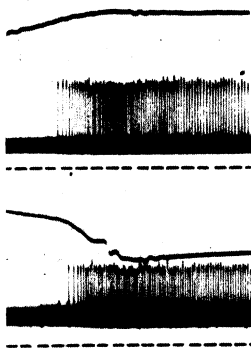


Fig. 8

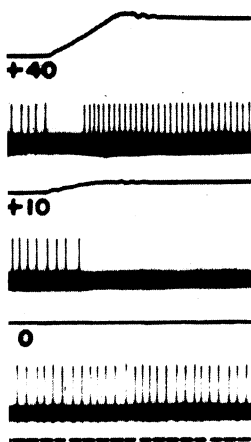


Fig. 9

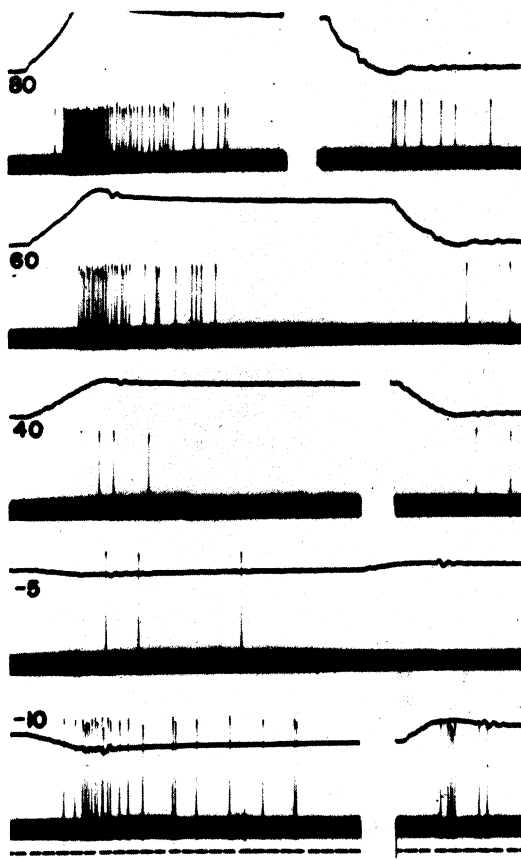


Fig. 10

Fig. 8. Discharge from a slowly adapting receptor which responds to both inflation (30 cc.) and forced deflation (20 cc.).

Fig. 9. Impulses from a slowly adapting receptor whose activity ceases only during small inflations above the expiratory level. Volume changes as indicated in cubic centimeters.

Fig. 10. Response of a rapidly adapting receptor to the indicated volume changes from the expiratory level. In most cases this receptor also responded when the original volume was restored 2 to 4 seconds later. Volume changes as indicated in cubic centimeters. (The action potentials are larger in the lower records because of progressive drying of the fine nerve strand during observations.)

the chest wall removed about one-third of the slowly adapting endings and about two-thirds of the rapidly adapting endings could be stimulated by withdrawing 20 cc. from the lungs at the end of expiration. Withdrawal of this same amount caused no activity in the remaining fibers.

Illustrations of slowly and of rapidly adapting endings which respond to both inflation and deflation are given in figures 8 and 10. Figure 9 is an example of a

slowly adapting ending which was out of action only at a volume slightly above the expiratory level under the particular experimental conditions. Other slowly adapting receptors continued to discharge impulses at every possible lung volume, exhibiting only a minimum of activity at some intermediate level (e.g., fig. 7, curve 2). The majority of the pulmonary receptors behaved more simply, however, and were either out of action at the end of expiration or could be stopped by a further small deflation.

In our experiments every fiber which was found to respond to deflation could also be stimulated by inflation. This contrasts with Adrian's conclusion that "...there is no doubt that deflation calls a new set of endings into play." We have certainly not made an exhaustive search for fibers stimulated only by deflation, because we were primarily interested in the response to inflation. Nevertheless, our procedure was such that we would expect to have recorded from some of them unless they were considerably smaller or much less numerous than the fibers stimulated by inflation.

Among the fibers which respond to both inflation and deflation almost all degrees of sensitivity to deflation have been observed. This is shown in figure 11

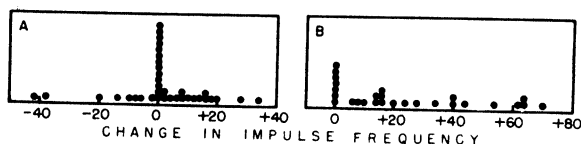


Fig. 11. Response of receptors to forced deflation with chest wall removed. The abscissa of each point represents the response of a single afferent fiber to withdrawing 20 cc. of air below the expiratory level. A. Slowly adapting endings. B. Rapidly adapting endings.

which gives the distribution of responses of slowly adapting endings (A) and rapidly adapting endings (B) to withdrawal of 20 cc. of air. The responses of the slowly adapting endings range from a considerable increase in impulse frequency from some receptors to a large decrease in frequency from others. The rapidly adapting receptors also exhibit a widely varied sensitivity to deflation although none of this kind can decrease significantly in activity because at the control level their ability to adapt has already caused them to drop out of action or discharge only occasionally with the cardiac pulse. The most significant feature of these distributions is that there is no separate group of receptors distinguished by a large response to deflation. This suggests that none are specialized for reporting deflation of the lungs.

In relating these findings to the normal physiology of the animal, it should be noted that removal of the chest wall appears to permit the lungs to collapse more completely during passive expiration than they do normally. This suggests that under more natural conditions any particular volume change from the expiratory level would elicit a larger response on inflation and a smaller response on deflation than those observed in our experiments, at least for the slowly adapting receptors. The rapid adaptors would presumably be less affected by the abnormal lowering of the expiratory volume, since they appear to adjust themselves

to any maintained volume. For example, many of this kind were stimulated not only on inflation and deflation, but also on return to the expiratory level (fig. 10).

DISCUSSION. *Reflex action of impulses from the two kinds of pulmonary receptors.* The reflex action of impulses from the receptors described above may be deduced by comparing the characteristics of the receptors with the two respiratory responses evoked by inflation of the lungs.

1. Small inflations of the lungs produce an inhibition of inspiration which may persist several seconds if inflation be maintained. The low threshold and long duration of this reflex can be accounted for by assuming that it is the result of impulses from the slowly adapting endings described above. Impulses from these receptors are, therefore, assumed to inhibit inspiration. This is in complete agreement with Adrian's interpretation.

2. Large inflations of the lungs result in a short-lasting inspiratory effort as described in a preceding paper (Larrabee and Knowlton, 1946). This brief inspiration is followed by inhibition of inspiration, if the lungs are kept distended. The evoked inspiration is well explained if impulses from the high threshold, rapidly adapting endings are assumed to excite inspiration. The striking similarity in time course of the discharge over a typical afferent and a typical efferent fiber is shown in figure 12. The inhibition which follows the evoked discharge is presumably due to impulses from slowly adapting endings which continue after the activity of the rapid adaptors has ceased.

If inspiration is thus assumed to be inhibited by impulses from slowly adapting endings and excited by impulses from rapidly adapting endings, then stimulation of inspiration by forced deflation of the lungs is also explained. The forced deflation would be signaled to the central nervous system by *a*, a decreased frequency of impulses from most of those slowly adapting fibers which remain in action throughout eupneic respiration, and by *b*, a burst of impulses from rapidly adapting receptors, the majority of which respond to both inflation and deflation. According to the foregoing analysis of the reflex actions of the two kinds of receptors, each of these changes in the afferent discharge would tend to cause inspiration, the first by reduction of inhibition, the second by increased excitation.

It has been said that forced deflation also stimulated about one-third of the slowly adapting receptors. This, in contrast to *a* and *b* above, would be expected to inhibit inspiration. Even with the chest wall removed, however, slow adaptors stimulated by deflation are in the minority, and reasons have already been given for believing that the number is still less under more natural conditions. It is therefore reasonable to assume that the two responses enumerated in the preceding paragraph normally constitute the most significant changes in the afferent discharge when the lungs are forcibly deflated.

Thus the various reflexes produced by inflation and deflation of the lungs are adequately accounted for by the two kinds of receptors here described.

Rôle in normal respiration. Most of the foregoing conclusions are based on the results of experiments with the chest wall removed. Having thus determined the reflex rôle of the various afferent fibers, it is now possible to consider how their action will modify the respiratory movements of an intact animal.

During eupneic respiration it is chiefly the inspiration-inhibiting (slowly adapting) fibers which are brought into action. They serve to limit the depth of in-

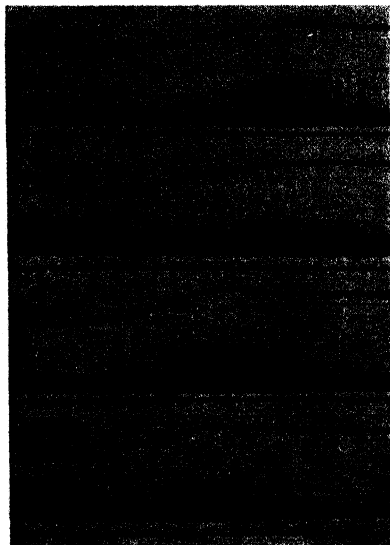


Fig. 12

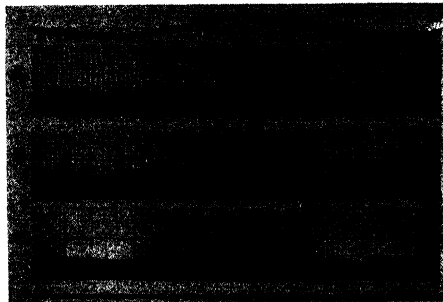


Fig. 13

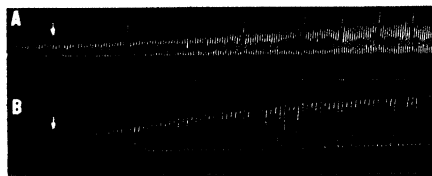


Fig. 14

Fig. 12. Comparison of the discharge over a vagal fiber from a rapidly adapting receptor (A and C) with the discharge reflexly evoked from a single phrenic motoneurone (B and D). Inflations of 60 cc., starting at the arrows, are made slowly in A and B, more rapidly in C and D. A and C (sensory) from one animal, B and D (motor) from another, both with chest wall removed. The respiration pump was stopped in expiration just before the start of each record. At the beginning of the motor nerve records there appears the end of a spontaneous discharge which is inhibited as the lungs are distended. Coinciding with the afferent discharge at each rate of inflation is another group of motor nerve impulses which is reflexly evoked by the distension of the lungs. (For further details concerning the motor discharge, confer Larrabee and Knowlton (1946).

Fig. 13. Impulses from a slowly adapting receptor during hyperpnea caused by rebreathing expired air. Cat breathing naturally with chest wall intact. Respiration recorded by pneumograph bag strapped around chest and abdomen (inspiration upwards). A, taken at start of rebreathing, B and C at intervals later. The afferent discharge during expiration becomes reduced as hyperpnea develops.

Fig. 14. Respiratory movements during hyperpnea caused by rebreathing expired air with vagus nerves intact (A) and cut (B). Respiration measured by volume recorder connected to a closed chamber containing the cat, the tracheal cannula being opened to the atmosphere. Animal breathing naturally with chest wall intact. Time in seconds. Arrows indicate start of rebreathing. The occasional extra-deep inspirations develop only when the vagus nerves are intact. There is no reduction of expiratory volume under either condition.

spiration and consequently to accelerate the respiratory rate in the manner described by Adrian (1933). The thresholds of the inspiration-exciting (rapidly adapting) fibers are so high that few are stimulated within the eupneic volume

range and the impulses from these few may not be enough to have a significant central effect.

An interesting property of the *slowly adapting endings* is their shifting threshold. This is illustrated in figure 13 which shows the discharge from a slowly adapting ending during several stages of hypernea induced by rebreathing the expired air. At the start impulses are discharged, with a varying frequency, throughout the entire respiratory cycle (A). As respiration deepens and the impulse frequency during inspiration increases, the discharge during expiration becomes reduced (B). Finally, there develops an interval of about one-half second during expiration in which no impulses are discharged (C). This phase of reduced activity was recognized by Adrian who pointed out its significance in shortening the expiratory pause. The pause is shortened because the fewer afferent impulses are less effective in delaying the onset of the next inspiratory discharge. Such an interpretation is in agreement with Scott's (1908) finding that the respiratory rate increases markedly during CO₂ hyperpnea only when the vagi are intact. (Cf. also fig. 14 and Adrian, 1933.) The altered afferent discharge is, however, only one of several mechanisms for accelerating the respiratory rate, since rebreathing expired air will sometimes cause a moderate speeding of respiration even after the vagus nerves are cut (Pitts, 1942).

The principal cause of this reduced afferent discharge during expiration in hypernea must be an elevation of the volume threshold of the ending, since a cat does not empty its lungs more during hyperpnea than during eupnea (fig. 14A), at least under the conditions of these experiments. Experiments with the chest wall removed indicate that distention of the lungs is indeed succeeded by a period in which the volume threshold of the receptors is elevated. Immediately following a prolonged inflation, after the lungs have been returned to their original volume, fibers which normally remain in action at the expiratory level discharge at a reduced frequency (fig. 15), and fewer impulses are elicited from any ending by injecting a given volume of air (fig. 16). Similar after-effects of prolonged stretch have been shown for carotid sinus pressure receptors (Bronk and Stella, 1935) and tension receptors in muscle (Matthews, 1933). These phenomena may be due in part to the reduced irritability which has been shown to follow intense activity in many kinds of nerve cells. In the case of the lungs, however, there is in addition a reversible yielding to stretch of the various tissues of which the lungs are composed. This is shown by a gradual decline in pressure when the lungs are held distended at constant volume (e.g., figs. 1, 15), by a drop in pressure below the initial value when the original volume is restored (figs. 10, 15), and by the fact that injection of a given volume of air produces a smaller rise in pressure when injected immediately after a period of distention (fig. 16). As a consequence of this yielding of lung tissue, the distorting force transmitted to the end organs is presumably reduced. Thus a reduction of both the intensity of the stimulus and of the irritability of the end-organ appear to contribute to the shift in the volume threshold of the pressure receptors.

The *rapidly adapting receptors* come into action in appreciable number only

when the depth of inspiration is increased. Even with a moderately large increase in depth, however, the discharge consists of not more than a few impulses occurring at the peak of some of the inspirations. Much greater activity is obtained in the very deep inspirations that occur under certain special conditions. For example, it is characteristic of the respiratory movements caused by high CO_2 or low O_2 in the anesthetized cat or dog that an occasional very deep inspiration appears (fig. 14). These inspirations are accompanied by an intense discharge from the rapidly adapting endings (fig. 17). Remembering that impulses from these receptors have been identified as excitator to inspiration, it appears likely that they are not only a result, but also a cause of the very deep inspirations.

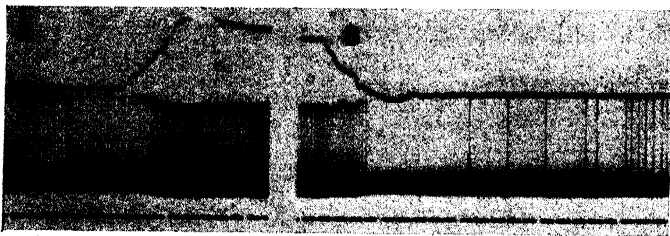


Fig. 15. Elevation of volume threshold after a prolonged inflation. A slowly adapting ending which was in activity at start of record dropped out of action temporarily when the initial volume was restored. Chest wall removed.

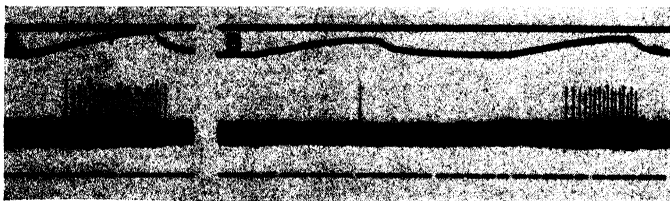


Fig. 16. Elevation of volume threshold after an inflation maintained for $3\frac{1}{2}$ seconds. A receptor which normally discharged over 25 impulses in response to the stroke of the respiration pump (A), discharged only one impulse in response to the same inflation (in B) 1.2 seconds after the prolonged inflation.

For it may be assumed that as the successive inspirations become progressively deeper during the development of hyperpnea, a level is eventually reached which suffices to activate a significant number of these excitator fibers. When this occurs the inspiration in progress can be expected to be profoundly modified. For the motor discharge to the muscles of inspiration will be reflexly increased and the lungs will be further distended, initiating in turn more afferent excitator impulses. In this connection it is important to remember that when the lungs are deeply inflated, the excitator reflex is of adequate intensity temporarily to break through the inhibition simultaneously produced by impulses from the slowly adapting endings (Larrabee and Knowlton, 1946). Thus the afferent excitator impulses can initiate an autogenous reflex cycle which, once started, tends to perpetuate

itself and may finally develop a maximal inspiration. Expiration ensues when the inspiration has been held long enough for end-organ adaptation to curtail the afferent excitator discharge or when other limiting factors are imposed upon the reflex. Following a deep inspiration, the volume threshold of the endings will remain elevated for a time due to reasons described in the preceding paragraph. This explains why another deep inspiration does not occur for several respiratory cycles (fig. 14A). In support of these explanations of the respiratory pattern during hyperpnea, it has been found that the occasional deeper inspirations do not appear after the vagus nerves have been cut (fig. 14B).

The very deep inspirations recur too infrequently during hyperpnea to contribute much to the minute volume, although even a small increase may be of importance to an individual in extreme respiratory distress. Of greater interest is the fact that impulses discharged from the rapidly adapting receptors can also reinforce deep inspirations under other circumstances. Consequently a very deep inspiration can be evoked by a stimulus whose intensity would cause an



Fig. 17. Impulses discharged from a rapidly adapting receptor during hyperpnea produced by rebreathing. This receptor was entirely inactive during normal quiet respiration. Animal breathing naturally with chest wall intact. Respiration recorded by bag pneumograph.

inspiration of only moderate depth were it not for this vagal reflex. Isolated deep inspirations occur involuntarily in sneezing, coughing, yawning, and sighing, as well as voluntarily in speaking and singing. A possible mechanism for reinforcing the initial deep inspiration in each of these acts is provided by the autogenous inspiratory reflex.

SUMMARY

The responses of single afferent fibers of the vagus nerve to changes in lung volume were recorded in cats under Dial anesthesia.

The fibers could be divided into two distinct groups according to the rate at which their end-organs adapted to lung inflation. The slowly adapting receptors had on the average a lower inflation threshold than the rapidly adapting receptors. Some receptors of both kinds responded to forced deflation of the lungs, but none were found which responded *only* to deflation.

The conduction velocity, measured in 20 fibers and corrected to body temperature, was between 8 and 44 meters per second.

The differences in adaptation and threshold of the two afferent fiber groups

when compared with the two reflexes evoked by inflation of the lungs, suggest that impulses from slowly adapting endings inhibit inspiration, while those from the rapid adaptors excite inspiration. This evaluation of function adequately accounts for the respiratory responses to both inflation and deflation of the lungs.

Only the slowly adapting fibers are in action in eupnea. A change in their threshold contributes to the increased rate of respiration in hyperpnea. The principal function of the rapidly adapting fibers appears to be a reinforcement of depth of certain deep inspirations which have been initiated through other mechanisms.

REFERENCES

- ADRIAN, E. D. *J. Physiol.* **79**: 332, 1933.
BRONK, D. W. AND G. STELLA. *This Journal* **110**: 708, 1935.
CATTELL, McK. AND H. GRUNDFEST. *Science* **81**: 645, 1935.
GASSER, H. S. p. 173 In ERLANGER and GASSER. *Electrical signs of nervous activity.* Philadelphia, Univ. of Pa. Press, 1937, 205 pp.
GASSER, H. S. AND H. GRUNDFEST. *This Journal* **127**: 393, 1939.
GESELL, R. *Ergeb. d. Physiologie* **43**: 477, 1940.
HEINBECKER, P. AND J. O'LEARY. *This Journal* **106**: 623, 1933.
LARRABEE, M. G. AND G. C. KNOWLTON. *This Journal* **147**: 90, 1946.
MATTHEWS, B. H. C. *J. Physiol.* **78**: 1, 1933.
PARTRIDGE, R. *J. Cell. and Comp. Physiol.* **2**: 367, 1933.
PITTS, R. *J. Neurophysiol.* **5**: 403, 1942.
SCOTT, F. H. *J. Physiol.* **37**: 301, 1908.
TSAL, C. *J. Physiol.* **73**: 382, 1931.
WORZNIAK, J. J. AND R. GESELL. *This Journal* **126**: p. 658, 1939.

THE METABOLIC REDUCTION AND NEPHROTOXIC ACTION OF TETRATHIONATE IN RELATION TO A POSSIBLE INTER-ACTION WITH SULFHYDRYL COMPOUNDS

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In a previous communication it was shown that in the dog the clearance ratio of creatinine and thiosulfate is unity. It was deemed of interest, therefore, to study the mechanisms whereby other oxides of sulfur are excreted by the kidney. For this purpose the immediate oxidation product of thiosulfate, namely, tetrathionate, was first selected. Two facts concerning tetrathionate, hitherto unreported, were immediately apparent: 1, tetrathionate is rapidly reduced to thiosulfate *in vivo*; 2, tetrathionate is toxic to the renal tubule to a degree that moderate doses produce complete anuria within less than an hour. The following report is concerned with the mechanisms by which the reduction of tetrathionate can be effected *in vivo* and its possible relationship to the nephrotoxic action.

Chemistry. References to the toxicity of tetrathionate are few and inconsistent. It has tacitly been assumed in clinical work that tetrathionate is no more toxic than thiosulfate (1). Chen, Rose and Clowes (2) reported the lethal dose of sodium tetrathionate in the dog to be 1000 mgm./kgm. Cacciavillani (3) observed a very much greater toxicity in the rabbit and emphasized the fact that the more pure the preparation the higher the toxicity. Inasmuch as the results to be reported below indicate that the toxicity of sodium tetrathionate may be related to its metabolic reduction to thiosulfate, the importance of working with a preparation that is chemically pure and not largely contaminated with thiosulfate is obvious. For these reasons the methods of preparation and analysis of the tetrathionate used in these studies and the analytical procedures for following metabolic conversion are briefly reviewed.

Preparation of sodium tetrathionate. Highly pure samples of sodium tetrathionate may be conveniently prepared by the dropwise addition of a concentrated solution of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ (250 grams in 150 cc. H_2O) to an alcoholic solution of iodine (127 grams I_2 and 50 grams NaI in 500 cc. of 90 per cent ethanol) (4, 5). The reaction mixture is vigorously stirred and maintained below 20°C . during the addition of thiosulfate. Precipitation of tetrathionate begins when approximately $\frac{1}{2}$ of the thiosulfate has been introduced. The reaction is considered complete when the color of the iodine solution attains a straw-like hue. One liter of absolute ethanol and 500 cc. of anhydrous ethyl ether are then added to the reaction mixture. After precipitation appears complete, the deposit is collected on a Buchner funnel and washed with small portions of absolute ethanol to remove excess iodine. The

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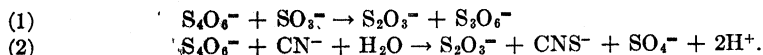
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precipitate is then sucked dry of excess alcohol and dissolved in an equal weight of H_2O . The solution is filtered by gravity through paper and the filtrate allowed to fall directly into 1 liter of absolute ethanol. The precipitate so formed is collected on a Buchner funnel, washed well with absolute ethanol, and dried at room temperature to constant weight *in vacuo* over CaCl_2 . The yield is about 100 grams of a white, crystalline material which is easily dissolved in an equal weight of water to form clear, non-turbid solutions with no iodine uptake.

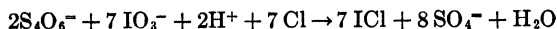
According to earlier reports sodium tetrathionate crystallizes out of ethanol-water as the hydrated salt, $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$ (5). The present compound also appears to contain 2 molecules of H_2O since on cautious drying at 80°C ., to avoid decomposition of the tetrathionate moiety of the molecule, a 11.4 to 11.8 per cent weight loss occurs. The theoretical value for the loss of 2 molecules of H_2O is 11.8 per cent. Upon analysis of samples which have been dried to constant weight at elevated temperatures 98.9 to 101.5 per cent of the expected tetrathionate activity of anhydrous $\text{Na}_2\text{S}_4\text{O}_6$ is obtained.

Analysis of sodium tetrathionate. Three methods were used to analyze the tetrathionate content of the hydrated salt. These depend, respectively, upon the reaction of the tetrathionate ion with sulfite, cyanide, and iodate. The first two reactants have been used in analytical procedures (6) and depend upon the following reactions:



In both reactions one mol of $\text{S}_2\text{O}_3^{2-}$ is quantitatively formed from each mol of $\text{S}_4\text{O}_6^{2-}$ and the iodine uptake of the resultant products provides a direct measure of the original content of $\text{S}_4\text{O}_6^{2-}$. (In the reaction with SO_3^{2-} excess HCHO is added to bind free SO_3^{2-} before titrating with iodine.) Various samples of the tetrathionate used herein were found to analyze by the sulfite and cyanide procedures as 98.8 to 102 per cent of $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$.

In the analysis of tetrathionate by reaction with iodate advantage is taken of the fact that iodate quantitatively oxidizes tetrathionate in the presence of HCl to sulfate (7). The overall reaction may be written as follows:



For the determination, 25 cc. of 0.1 N KIO_3 are added to 25 mgm. samples of tetrathionate followed by 5 cc. of 2N HCl . After 5 minutes 5 cc. of 10 per cent KI are added to convert ICl and unreacted IO_3^- to free iodine which is then titrated against 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$. In order to calculate the tetrathionate content of the sample analyzed cognizance is taken of the fact that in conventional iodimetry 1 mol of $\text{Na}_2\text{S}_2\text{O}_3$ is equivalent to the iodine liberated by the reaction of $\frac{1}{2}$ mol of IO_3^- with an excess of iodide. By comparison the above equation reveals that 1 mol of $\text{S}_4\text{O}_6^{2-}$ reacts with an amount of IO_3^- equivalent to 21 mols of $\text{S}_2\text{O}_3^{2-}$, with the production of ICl equivalent to 7 mols of $\text{S}_2\text{O}_3^{2-}$. Thus, the content of $\text{Na}_2\text{S}_4\text{O}_6$ in the samples analyzed may be calculated from the results of the titration as follows:

$$\text{mgm. Na}_2\text{S}_4\text{O}_6 = \text{cc. titration } \Delta \times \frac{27}{14}$$

Various samples analyzed by the iodate reaction were found to be 98.9 to 99.4 per cent $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$.

Determination of tetrathionate and thiosulfate in plasma and urine. The methods for the determination of thiosulfate in plasma and urine have been previously reported (8). For the simultaneous determination of tetrathionate and thiosulfate, advantage is taken of the fact that whereas both ions are oxidized by iodate to sulfate, only thiosulfate is oxidized by iodine. The determination in plasma is as follows: tungstate filtrates are prepared as previously described (8). Recovery of tetrathionate from tungstate filtrates is complete at levels of 15 mgm. per cent or higher. One aliquot of the filtrate is made slightly alkaline to

phenolphthalein, following which iodate, potassium iodide, and hydrochloric acid are added in the order named, and thiosulfate determined and calculated as previously described (8). The other aliquot is reacted with potassium iodate in the presence of hydrochloric acid before the addition of potassium iodide and the total thiosulfate and tetrathionate content determined as $\text{Na}_2\text{S}_2\text{O}_4$. This procedure is identical with the one previously described for the determination of thiosulfate in plasma by means of its reaction with iodate (8). The tetrathionate content of the sample of plasma is then calculated in the following manner:

$$\text{mgm. Na}_2\text{S}_4\text{O}_6 = (B-A) \frac{270}{158} \times \frac{8}{14}$$

where A = mgm. $\text{Na}_2\text{S}_2\text{O}_4$ determined by analysis of iodine uptake of samples of filtrate and B = mgm. $\text{Na}_2\text{S}_2\text{O}_4$ calculated from the reaction of samples of filtrate with iodate.

The same procedure can be applied directly to urine which has been appropriately diluted.

Conversion of tetrathionate to thiosulfate in vivo. The conversion of tetrathionate to thiosulfate was observed in rabbits and dogs. Pure samples of tetrathionate were injected intravenously and the tetrathionate and thiosulfate content of the blood determined at various intervals. The results are presented in tables 1 and 2. In the rabbit following the injection of 500 mgm./kgm. of $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$ conversion occurred to the extent that blood levels of approximately 70 mgm. per cent of $\text{Na}_2\text{S}_2\text{O}_4$ are attained within 30 minutes. Conversion rapidly proceeds so that only traces of tetrathionate are present after four hours. In the dog the rate of conversion appears to be somewhat slower, but nevertheless is practically complete within 4 hours.

It is immediately apparent that whereas the normal rabbit (table 1) and the normal dog (8) rapidly excrete thiosulfate, in both species the thiosulfate which results from the reduction of tetrathionate *in vivo* is largely retained. This is due to the severe nephrotoxic action of tetrathionate which in the dosage employed rapidly causes complete anuria.

In view of the highly nephrotoxic action of tetrathionate it was of interest to determine whether the kidney plays a major rôle in its reduction. The data in table 1 indicate that whereas the nephrectomized rabbit may have a somewhat higher blood level of tetrathionate 30 minutes after administration than control animals which retain renal function for a limited period, nevertheless the rates of reduction in the two groups are comparable.

The observations in nephrectomized rabbits afforded an opportunity to calculate the volume distribution of tetrathionate. On the basis of the blood levels of tetrathionate and thiosulfate 30 minutes after injection the apparent volume distribution in five rabbits was in 23, 19, 26, 24 and 22 per cent of the body weight, respectively. Thus, like thiosulfate (8), tetrathionate appears to be distributed in the extra-cellular fluid. This fact is of importance in considering the site of metabolic reduction (*vide infra*).

Inasmuch as the analytical procedure employed in demonstrating the conversion of tetrathionate to thiosulfate *in vivo* depends on the iodine uptake of thiosulfate the remote possibility exists that some other metabolic product of tetra-

TABLE 1
Reduction of tetrathionate to thiosulfate in the rabbit

DOSE ADMINISTERED INTRAVENOUSLY	RABBIT	TIME AFTER INJECTION	ANALYSIS OF PLASMA CONSTITUENTS	
			Na ₂ S ₂ O ₃	Na ₂ S ₄ O ₆
<i>mgm./kgm.</i>		<i>min.</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
500 Na ₂ S ₄ O ₆ ·2H ₂ O	1	35	72.5	60.0
		240	146	11.7
	2	30	64.5	119
		240	138	3.9
	3	29	75.0	103
		221	146	5.9
	4	45	75.6	75.5
		225	106	5.9
	5	45	141	30.2
		210	151	10.7
	6	75	145	21.4
		200	133	1.0
500 Na ₂ S ₄ O ₆ ·2H ₂ O in nephrectomized animals	7	31	65.5	133
	8	30	88.4	151
	9	30	51.5	123
		227	146	9.8
	10	30	59.0	138
	11	30	49.1	157
		215	172	13.7
500 Na ₂ S ₂ O ₃	12	33	137	
		240	4.1	
	13	29	111	
		246	6.5	
	14	40	97	
		210	2.7	
	15	60	84.5	
		210	12.4	

thionate such as sulfite or sulfide is being formed. However, chemical proof to identify the conversion product as thiosulfate is obtained in those experiments in

which conversion of tetrathionate to thiosulfate is practically complete. In this circumstance, identical analytical results are obtained for the determination of thiosulfate based upon its oxidation by iodine or iodate (table 2, dogs 3, 4, 6, and 8; table 1, rabbit 6).

Nephrotoxic action of tetrathionate. Following the intravenous injection of tetrathionate in anesthetized, catheterized dogs, anuria develops within 30 to 60 minutes. Moreover, if renal function is quantitatively followed by means of creatinine clearance, it is evident that renal impairment follows immediately

TABLE 2
Reduction of tetrathionate to thiosulfate in the dog

INTRAVENOUS DOSE	DOG	TIME AFTER INJECTION	ANALYSIS OF PLASMA CONSTITUENTS	
			Na ₂ S ₂ O ₃	Na ₂ S ₄ O ₆
<i>mgm./kgm.</i>		<i>min.</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
725	1	30	41.8	186
		210	200	16.6
500	2	30	28.0	118
		207	126	7.8
	3	29	53.1	70.4
		214	94.4	0.0
	4	32	53.5	88.6
		219	118	0.0
	5	32	49.5	73.0
		216	31.6	6.5
	6	32	44.3	88.8
		212	114	1.0
	7	14	21.3	172
		178	1.6	13.7
300	8	13	10.7	77.5
		265	43.7	0.7

after the injection of tetrathionate and becomes progressively more severe. Such an experiment is depicted in table 3, in which is presented the rapid development of complete renal shutdown associated with conversion of tetrathionate to thiosulfate.

The nephrotoxic action as well as the order of toxicity of sodium tetrathionate has been further demonstrated in experiments on dogs and rabbits in which the lethality of tetrathionate has been related to its effect on renal function as evidenced by serum urea concentration. As can be seen from table 4, death from

TABLE 3

The effect of tetrathionate on renal function and its reduction to thiosulfate in an anesthetized dog

Female, 19.2 kgm., under pentobarbital anesthesia; 0 to 5 min. 6 grams of creatinine and 10 grams mannitol in 300 cc. of water and 47 to 50.5 min. 11 grams $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$ in 220 cc. H_2O infused intravenously.

TIME	INTRAVENOUS INFUSION	URINE VOLUME	CREATININE		$\text{Na}_2\text{S}_4\text{O}_6$ IN PLASMA	$\text{Na}_2\text{S}_2\text{O}_3$ IN PLASMA
			Concentration in plasma	Filtration rate		
min.		cc.	mgm. per cent	cc./min.	mgm. per cent	mgm. per cent
0	Creatinine and mannitol					
30			47.4			
45	$\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$	58	37.0	69.3		
61		58		38.7		
75		27	31.5	15.3	172	21.3
105		Anuric	31.1		130	38.0
165		Anuric	29.9		85.0	72.0
225		Anuric	30.2		27.4	141
			30.5		13.7	146

TABLE 4

The toxicity of tetrathionate administered intravenously to rabbits and dogs

SPECIES	DOSE OF $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$	MORTALITY	DAY OF DEATH	SERUM UREA N IN MGm. PER CENT			
				48 hrs.	72 hrs.	96 hrs.	144 hrs.
	mgm./kgm.						
Rabbit	1000	3/3	1 to 1½ hrs.				
	500	3/3	2, 2, 2				
	250	2/2	5, 7			187(2)	
	150	8/8	3, 3, 3, 4, 4, 5, 6, 7		165(8)		
	100	12/12	4, 4, 4, 5, 7, 7, 9, 10, 10, 10, 12, 14	89(2)	163(7)	200(2)	180(3)
	50	0/3			45(3)		36(3)
	25	0/3			11(3)		15(3)
Dog	750	1/1	3	142			
	660	1/1	2				
	500	6/6	4, 4, 6, 6, 7, 9	126(6)		234(2)	
	250	1/2	7	57(2)		143(2)	
	125	0/1		48		82	

minimal lethal doses of sodium tetrathionate is delayed and associated with uremia. Furthermore, pathological examination of the kidney reveals severe

necrotizing lesions of the proximal tubule. Of extreme interest is the fact that in the dog evidence of advanced necrosis is apparent within 30 minutes.⁴

Following minimal lethal doses of tetrathionate animals exhibit little or no acute signs of intoxication and at death significant pathological findings are limited to the proximal renal tubule. However, supralethal doses (500 mgm./kgm. or more) in the dog cause hyperpnea, vomiting and a peculiar stiffening of the hind legs which results in an ataxia which may persist for several days. In rabbits the muscular effects are even more prominent. In this species muscular contraction occurs readily but relaxation is delayed. This phenomenon has not been further analyzed.

The in vitro conversion of tetrathionate to thiosulfate. In order to explain the mechanism of the conversion of tetrathionate to thiosulfate *in vivo* studies on the reduction of tetrathionate *in vitro* were performed. It was observed that a variety of sulfhydryl compounds could rapidly convert tetrathionate to thiosulfate. The following reaction was postulated:



To prove this reaction the behavior of tetrathionate with 2,3-dimercaptopropanol (BAL), 1-thiosorbitol, cysteine and glutathione was followed in some detail. In table 5 are shown the electrometric titrations of hydron made available from the various mercaptans in the presence and absence of tetrathionate. It is evident that 2 mols of hydrogen ion are produced for each mol of tetrathionate reacted, as expected from the above equation.

Proof of the formation of thiosulfate was complicated by the fact that the iodine uptake of the reacting mercaptan and the thiosulfate formed are equivalent. However, in the case of 2,3-dimercaptopropanol an opportunity for distinction was afforded due to the fact that in its oxidation by tetrathionate the insoluble disulfide is formed. This was removed by filtration. Iodimetric titration of aliquots of filtrate yielded theoretical values for thiosulfate according to the equation written above. The fact that the iodine uptake represented the oxidation of thiosulfate to tetrathionate was proved by the re-conversion of tetrathionate to thiosulfate with both sulfite and cyanide. The fact that thiols can reduce tetrathionate has also been demonstrated by Baernstein (9). Moreover it has been shown by Anson (10) that tetrathionate can oxidize —SH groups of denatured egg albumin.

The prophylaxis of tetrathionate poisoning with mercaptans. That tetrathionate can readily react with mercaptans *in vivo* is shown by experiments in which rabbits were completely protected from the lethal and nephrotoxic action of LD100 doses of tetrathionate by the prophylactic administration of 1-thiosorbitol or cysteine. The completeness of such protection as evidenced by failure to observe any rise in serum urea nitrogen is shown in table 6.

Evidence for the rapidity with which tetrathionate damages the renal tubule was obtained from experiments in which the administration of 1-thiosorbitol or

⁴ We are indebted to Major A. M. Ginzler for the pathological study on these animals. A more detailed analysis of the renal pathology will appear in a separate paper.

cysteine was delayed (table 6). Despite the fact that both thiols can effect a rapid conversion of tetrathionate to non-toxic thiosulfate, rabbits were not benefited when treatment was delayed for fifteen minutes. Moreover no protection was afforded by the subsequent injection of glutathione.

DISCUSSION. Several conclusions and inferences can be drawn from the above experiments. It has been observed that tetrathionate can be reduced to thio-

TABLE 5

Titrateable acidity of aqueous solutions of various mercaptans following addition of $\text{Na}_2\text{S}_4\text{O}_6$

Sulfhydryl activity of mercaptans determined iodimetrically; $\text{Na}_2\text{S}_4\text{O}_6$ (0.625 molar) added stoichiometrically to combine with available $-\text{SH}$; molar equivalents of NaOH (0.507 molar) calculated with respect to available $-\text{SH}$; pH determined by glass electrode (Beckman pH-Meter).

MERCAPTAN	AMOUNT OF MERCAPTAN- TITRATED	CONCENTRATION C.F. $-\text{SH}$	pH	EQUIVALENTS OF NaOH ADDED	
				In absence of $\text{Na}_2\text{S}_4\text{O}_6$	In presence of $\text{Na}_2\text{S}_4\text{O}_6$
BAL (2,3-dimercapto- propanol)	m. Mols 1.34	molar 0.107	4.0		0.91
			5.0		0.96
			6.0	0.00	0.98
			7.0	0.01	0.99
			8.0	0.03	1.00
l-thiosorbitol	2.49	0.050	4.0		0.41
			5.0		0.71
			6.0	0.00	0.88
			7.0	0.00	0.97
			8.0	0.03	1.00
Cysteine-HCl	2.49	0.050	4.0	0.90	1.72
			5.0	0.91	1.82
			6.0	0.92	1.85
			7.0	0.97	1.88
			8.0	1.22	1.98
Glutathione*	0.25	0.0099	4.0	0.63	0.94
			5.0	0.92	1.67
			6.0	0.96	1.89
			7.0	0.98	1.91
			8.0	1.09	1.97

* Titrated with 0.101 N NaOH ; $\text{Na}_2\text{S}_4\text{O}_6$ added as 0.1 M.

sulfate *in vivo* and coincidentally renal failure occurs. It has also been observed that tetrathionate can oxidize mercaptans *in vitro*. Moreover, that a reaction between tetrathionate and mercaptans can readily occur *in vivo* is evident from the protective action afforded by l-thiosorbitol and cysteine. It is, therefore, reasonable to attribute the reduction of tetrathionate *in vivo* in the absence of extraneous mercaptan to the oxidation of $-\text{SH}$ normally present in the body.

The above facts afford explanation for the nephrotoxic action of tetrathionate. It is known that heavy metals, capable of inhibiting SH-containing enzymes, by the formation of mercaptides, have a noteworthy toxic action on the kidney, damaging proximal tubules specifically. It is reasonable to expect, therefore, that a compound capable of oxidizing SH would also prove to be nephrotoxic.

The vulnerability of the kidney to mercury has been attributed to the fact that this organ is subjected to an unduly high concentration of Hg^{++} in the course of

TABLE 6

The effect of the intravenous administration of mercaptans on the toxicity of tetrathionate injected intravenously in rabbits

DOSE OF $\text{Na}_2\text{S}_4\text{O}_6 \cdot 2\text{H}_2\text{O}$ mgm./kgm.	THERAPY			DAY OF DEATH	SERUM UREA N IN MG. PER CENT		
	Mercaptan	Dose mgm./kgm.	Time min.		48 hrs.	72 hrs.	96 hrs.
150	1-thiosorbitol	400	-1	S*		12.6	
		400	-1	S		14.2	
		400	-1	S		10.2	
		400	-1	S		12.2	
100	1-thiosorbitol	500	+15	6	106		223
		500	+15	4	140		
		500	+15	3	109		
		500	+15	5	105		250
100	1-thiosorbitol†	500	+15	9		184	
		500	+15	S		83.8	
		500	+15	4		230	
		500	+15	8		196	
150	cysteine-HCl‡	400	-1	S		15.6	
		400	-1	S		11.6	
		400	-1	S		7.6	
		400	-1	S		22.3	
100	cysteine-HCl‡	400	+15	3			
		400	+15	9		212	
		400	+15	6		220	

*S = Survival.

† Injection of 1-thiosorbitol followed within 1 minute by 50 mgm./kgm. glutathione.

‡ Cysteine-HCl neutralized with NaOH (phenol red) before injection.

its excretion. The same explanation might apply to the peculiar sensitivity of the kidney to $\text{S}_4\text{O}_6^{--}$. However, the high susceptibility of the kidney to agents capable of acting upon —SH groups possibly may point to a unique dependence of the renal tubule on enzyme systems which contain thiol groups or are activated by thiol-containing compounds like glutathione (11).

On first thought one might presume that tetrathionate enters the renal tubular cell and at this site effects the inactivation of intracellular enzymes by the oxida-

tion of essential —SH groups. However, several facts presented above afford an alternative explanation. It has been shown that both tetrathionate and thiosulfate are distributed for the most part extracellularly. It is unreasonable to assume, therefore, that tetrathionate enters the cell, is reduced to thiosulfate, diffuses from the cell, and thereafter remains extracellular. Moreover, reduction of tetrathionate takes place readily in the absence of the kidney indicating that other organs are contributing to the metabolism of tetrathionate. It must be concluded, therefore, that for the most part the reduction of tetrathionate occurs extracellularly and is accomplished by —SH containing compounds capable of diffusing across cell membranes. In this manner cells could be largely depleted of diffusible —SH. Furthermore, in view of the large amounts of tetrathionate which can be metabolized, it is not unlikely to assume that —S—S— compounds resulting from extracellular oxidation can be reduced intracellularly and, thereby, afford a continuous source of —SH. The fact that tetrathionate can oxidize glutathione *in vitro* at physiological pH permits the implication of glutathione as the diffusible intermediate in this process.

The cataclysmic nature of the events occurring in the proximal tubule which result in irreversible cellular damage within 15 minutes points to a more severe metabolic disturbance than is usually associated with specific —SH inhibitors. Barron and Singer (11) have offered the hypothesis that glutathione has the important function of maintaining in the reduced state, which is essential to catalytic activity, the labile —SH groups of protein moieties found in many intracellular enzyme systems. On the basis of such a mechanism it is conceivable that the depletion of intracellular glutathione by extracellular reaction with tetrathionate could lead to simultaneous enzyme inactivation and consequently metabolic derangement at multiple loci within the cell.

If one postulates such a basic mechanism to account for the injury sustained by the cells of the proximal tubule, the question might well be raised as to why the cytotoxic action of tetrathionate is not more widespread. This is indeed an enigma which may favor the view that the action of tetrathionate on the proximal tubule is a direct one following penetration of the agent intracellularly. However, the data clearly show that large amounts of tetrathionate in extracellular fluid are capable of being reduced in nephrectomized animals, by substances contributed by tissues other than the kidney. One is thus left with two alternative conclusions. Either the proximal tubules of the kidney are uniquely permeable to tetrathionate and the loss of a diffusible reductant is without significant effect to both renal and extrarenal cells or else the proximal tubular cells are specifically sensitive to the loss of diffusible reductant.

The mechanism by which tetrathionate is capable of causing complete anuria within 30 minutes is also of interest. That no significant change in systemic blood pressure occurred has been demonstrated in experiments in which blood pressure was recorded continuously during the time that anuria developed. That anuria is due to afferent arteriolar constriction would seem unlikely due to its gradual onset and the fact that once established, urine flow does not spontaneously return. The rapidity with which histological evidence of damage

appears in the proximal tubule would support the view that anuria is due to swelling of the cells of the proximal tubule. In this connection it is of interest to note that after anuria develops, the intravenous injection of 3 cc./kgm. of a 30 per cent solution of NaCl with the purpose in mind of dehydrating the cells of the renal tubule re-establishes a flow of urine for a variable period. The urine obtained is replete with casts.

The biological reduction of tetrathionate to thiosulfate is not unique to mammalian species. Intestinal anaerobes have been reported readily to effect this conversion. The ability to reduce tetrathionate by *Bacterium paratyphosum* B has been attributed to an enzyme, "bacterial tetrathionase" (12). However, in view of the ready reduction of tetrathionate by thiols of natural occurrence, the possibility must be considered that the conversion in bacteria may be associated with the metabolic production of —SH compounds.

There is a tendency to make little distinction between tetrathionate and thiosulfate in so far as the therapeutic use of these agents is concerned. Thus, tetrathionate has been advocated in the treatment of thromboangiitis obliterans (1), and drug-house brochures suggest its applicability in all cases where thiosulfate may be of therapeutic value. Fortunately, the recommended doses are low. In view of the nephrotoxic action of tetrathionate its indiscriminate use as a medicinal should be discouraged.

SUMMARY AND CONCLUSIONS

In the rabbit and the dog tetrathionate is rapidly reduced to thiosulfate. Coincidentally there is observed a nephrotoxic action of such severity that in anesthetized, catheterized dogs complete anuria results within 30 to 60 minutes after the injection of moderate doses. Pathological examination reveals a discrete lesion of the cells of the proximal tubule which has advanced to the stage of nuclear degeneration within 30 minutes.

It is possible to reduce tetrathionate *in vitro* with 2,3-dimercaptopropanol, 1-thiosorbitol, cysteine, and glutathione with the stoichiometric formation of the corresponding disulfides. That thiols can react with tetrathionate *in vivo* is evident from the fact that the prophylactic administration of 1-thiosorbitol or cysteine protects against the nephrotoxic action of tetrathionate. Evidence is presented that the distribution and site of reduction of tetrathionate are extracellular.

Two alternative theories are presented that 1, tetrathionate selectively permeates the cells of the proximal tubule to exert a direct toxic action on catalytic systems dependent on the presence of —SH, or 2, the cells of the proximal tubules are selectively damaged by the removal of diffusible —SH compounds.

REFERENCES

- (1) THEIS, F. V. AND M. R. FREELAND. Arch. Surg. **40**: 190, 1940.
- (2) CHEN, K. K., C. L. ROSE AND G. H. A. CLOWES. Am. J. Med. Sci. **188**: 767, 1934.
- (3) CACCIAVILLANI, B. Boll. Soc. Ital. Biol. Sper. **11**: 756, 1936 (Chem. Abst. **31**: 2686, 1937).

- (4) MELLOR, J. W. A comprehensive treatise on inorganic and theoretical chemistry. London: Longmans, Green and Co., 1930, p. 610-620.
- (5) AUERBACH, F. AND I. KOPPEL. Abegg's Handbuck der anorganischen Chemie. Leipzig: Verlag von S. Hirzel, 1927, p. 541-585.
- (6) KURTENACKER, A. AND E. GOLDBACK. Ztschr. anorg. allgem. Chem. **166**: 177, 1927.
- (7) JAMIESON, G. S. Am. J. Sci. **39**: 639, 1915 (Chem. Abst. **9**: 1886, 1915).
- (8) GILMAN, A., F. S. PHILIPS AND E. S. KOELLE. This Journal **146**: 348, 1946.
- (9) BAERNSTEIN, H. D. J. Biol. Chem. **115**: 25, 1936.
- (10) ANSON, M. L. J. Gen. Physiol. **24**: 399, 1940.
- (11) BARRON, E. S. G. AND T. P. SINGER. Science **97**: 356, 1943.
- (12) POLLOCK, M. R. AND R. KNOX. Biochem. J. **37**: 476, 1943.

ELECTRICAL MANIFESTATIONS OF THE CEREBELLUM AND CEREBRAL CORTEX FOLLOWING DDT ADMINISTRATION IN CATS AND MONKEYS

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The compound, 2,2 bis (p-chlorophenyl) 1,1,1 trichlorethane (DDT) has marked effects on the nervous system of mammals. A number of investigators (Woodard, Nelson and Calvery, 1; Smith and Stohlman, 2; Bing, McNamara and Hopkins, 3; Philips and Gilman, 4) have noted or described the tremors, asynergia and convulsions following either the oral or intravenous administration of this compound. The portion of the nervous system attacked by DDT in producing this syndrome is not known but Bing, McNamara and Hopkins (3) have noted the similarity of some of the DDT symptoms to the syndrome of cerebellar deficiency. Lesions in some of the cerebellar nuclei after chronic administration of DDT have also been noted by Haymaker and Ginzler (5).

The present report is intended to be a descriptive summary of the effects of DDT as revealed by a study of the spontaneous electrical rhythm of the cerebral cortex and the cerebellum.

EXPERIMENTAL PROCEDURES. For this study 13 young monkeys (*Macaca mulatta*) weighing 2.0-2.5 kgm. and 24 cats (1.9-3.8 kgm.) were employed. The potentials were led off the cerebral cortex and cerebellum either by means of screw electrodes inserted into the calvarium or in the case of the exposed cerebral or cerebellar cortex by means of silver electrodes mounted within small lucite blocks which rested on the pial surface. Each "active" electrode was pitted against an "indifferent" lead which was either the right or the left ear. The "indifferent" lead was made the common ground for the several "active" electrodes, which were fed successively in groups of four into a four-channel electroencephalograph. The recording was accomplished with ink-writers having a linear response up to 80 c.p.s.

In preparing the animals light sodium pentobarbital anesthesia (15-20 mgm. per kgm.) was employed. In experiments where the effects of an anesthetic were to be avoided in order to bring out the convulsive action of DDT, ether was given, a tracheal cannula was inserted and all operative procedures were performed. Before beginning the experiment recovery from the anesthesia was permitted. To avoid the artifacts and difficulties associated with recording from an animal in tremor or in convulsions, curare (intocostin) was employed along with artificial respiration. Large doses of curare have an effect on the central nervous system (Pick and Unna, 6) but it was our experience that an injection of 4 mgm. per kgm. intramuscularly was sufficient to maintain the animals in a quiet condition for about an hour without influencing the pattern

of electrical activity from the brain. Additional injections of 1 to 2 mgm. per kgm. of curare were given during the course of an experiment whenever movements appeared. The design of the research was to record the spontaneous electrical activity from various regions of the cerebral cortex and cerebellum before the intravenous administration of DDT and at varying intervals thereafter. The DDT was injected in the form of either a lecithin emulsion (Philips and Gilman, 4) or a gum arabic emulsion (Hassett, 7). The emulsions contained 1 per cent DDT made up in isotonic saline or in Ringer solution.

RESULTS. 1. *Non-Convulsive Electrical Manifestations.* The convulsive episodes of DDT are readily prevented and the tremors prevented or reduced by the use of certain central nervous system depressants (Smith and Stohman, 2; Philips and Gilman, 4). In the present experiments it was found that small doses (15 mgm. per kgm.) of sodium pentobarbital prevented the appearance of periodic convulsive episodes. However, significant changes in the spontaneous electrical activity from the brain following DDT occurred in these animals. The most obvious alterations occurred in the rhythm recorded from the cerebellum. Typical of such changes in cats are those shown in figure 1, recorded by means of screw electrodes in the calvarium. The experimental cat (no. 1) was controlled by another cat (no. 2) which was similarly treated except that in place of the DDT emulsion, which was injected into the experimental animal, the control received an equal volume per kgm. of emulsion without the DDT. The pre-DDT records (I), as compared with the post-DDT electrograms (II) show clearly an increase in magnitude of the cerebellar rhythm after DDT as compared with the unchanged activity in the control animal. Another experiment is included in which electrograms from the experimental (no. 3) and the control (no. 4) are shown for the pre-DDT stage (I) and at two successive periods (II, III) following the administration of DDT into the experimental cat.

The spontaneous rhythm of the cerebral cortex of the cat was also significantly modified by the action of DDT. This alteration was not so much in the direction of a change in magnitude, though there were small increases in voltage (fig. 1, cat 1), but rather in the direction of a shift from a pattern with bursts of 8-12 c.p.s. waves, to a rhythm with an almost continuous and regular discharge at this frequency. This shift is obvious in the experiment (fig. 1) in which the cortical pattern in cat 3 before DDT (I) is compared with that recorded after the injection of this compound (II, III).

A progressive increase in the amplitude of the cerebellar activity following DDT was also obtained in monkeys (fig. 1, lower records B, C). This increase in amplitude did not continue indefinitely. One to two hours after the injection of DDT the cerebellar rhythm attained a constant peak voltage or even began to decrease. Such effects of DDT were obtained both with screw electrodes and with pial electrodes. The cortical potentials of the monkey were modified in several particulars by DDT. Not only did a regularization in pattern occur, though this was typically never as marked as in the cat, but the voltage increased slightly and characteristic large waves with rather spike-like qualities appeared. These waves are seen occurring in bursts in the post-DDT period in record 4A of

modified, but, in addition, periodic tonic-clonic electrical discharges were observed. In the discussion which follows, the term, pre-episode, will refer to the period from the moment of injection of the DDT to the beginning of the first periodic episode; the word, episode, will be employed in connection with the characteristic, periodic seizures to be described in the next section and the term, inter-episode, will be used in connection with the phases of the electrograms occurring between the end of one episode and the beginning of the next succeeding episode.

The typical effect of an intravenous dose of DDT between 50 and 75 mgm. per kgm. into non-anesthetized, curarized monkeys is illustrated in figure 2. There was first an increase in the electrical activity from the cerebellum (B, C) and the cortex (A) as in the case of lightly anesthetized monkeys. Well-differentiated (200–500 microvolts) spike-like waves eventually appeared and became increasingly prominent (records 3–6) in both the cerebellar and the cortical leads. The intervening activity between these waves gradually subsided and the spike-like waves, which for purposes of reference are called fast waves, were seen to stand out either as single waves or as groups of 2, 3 or even more (record 6). Certain significant characteristics of these fast waves are illustrated in figure 2.

1. The fast waves from the pre-motor cortex (area 6) were in complete temporal synchrony with fast waves from the cerebellum (pyramis and lobulus simplex). As will be shown later, the entire motor cortex (areas 4 and 6) was synchronized in this way with certain areas of the cerebellum.

2. Whereas the fast waves of the motor cortex were positive in sign (deflection down), the cerebellar fast waves were predominantly negative (deflection up) or somewhat diphasic with the negative phase occurring initially (fig. 2).

3. The cortical-cerebellar synchronization was not an experimental artifact for it was recorded from either the intact or the exposed brain. Areas other than the motor cortex and cerebellum revealed little or no fast wave activity. Since the animals were quiet, movement artifacts were not involved.

In non-anesthetized cats the changes in the electrical activity of the brain were not as progressively clear following the intravenous inject of DDT. The cerebellar rhythm increased but the cortical activity, except possibly for a slight increase in magnitude, presented no clearly differentiated modifications until fast waves appeared. These differed from the corresponding waves in monkeys in a number of characteristics. Some were predominantly monophasic and negative while others were diphasic, with the initial swing in the positive direction. There was little tendency for these pre-episodal fast waves to occur in groups as was the case in monkeys. As in monkeys, the fast waves of the cat were most prominent in cortical areas at or close to the motor cortex but fast wave synchronization between the motor areas and cerebellum, though present, was not prominent in cats.

B. Episodal electrical manifestations. In 60–90 minutes following DDT administration in monkeys, and in 20–90 minutes in cats, periodic episodes of electrical activity made their appearance. The first two to four episodes were

of atypically short duration (5-15 sec.) and quite atypical in pattern. After these were spent, seizures of a rather constant pattern and extending over a period of 30 to 120 seconds occurred at intervals of 2 to 20 minutes. As many as forty such episodes were recorded during a three hour period in one monkey. These seizures did not remain constant in pattern or duration over the entire period of occurrence. With successive seizures the inter-episodal intervals progressively increased, the episodal pattern suffered changes and the magnitude of the potentials as well as the duration of each seizure decreased. Because of space limitations it is not possible to present all the variations which occurred. A description will therefore be given of the typical episode occurring during the period of maximum development, which was about 2 to 3 hours following the

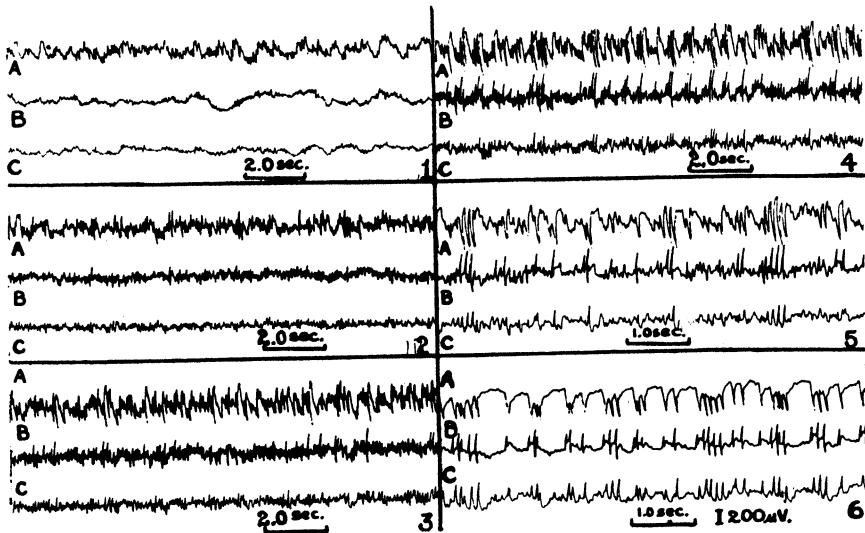


Fig. 2. Electrograms obtained by means of a screw electrode over the right motor cortex, area 6 leg (A) and by means of pial electrodes on the exposed pyramis vermis (B) and the exposed medial lobulus simplex (C) of a monkey. The records before DDT (1) are compared with those recorded 23 minutes (2), 31 minutes (3), 36 minutes (4), 39 minutes (5) and 43 minutes (6) after the intravenous injection of 60 mgm. per kgm. of DDT.

injection of DDT. It is to be understood that in the experiments the animals were paralyzed and no motor manifestations took place which could have affected the recordings.

In monkeys following the episode which preceded the seizure to be described, the cortical electrograms were relatively quiet and typically free of fast wave activity. Eventually fast waves appeared. At first small and of infrequent occurrence, they progressively increased in magnitude and frequency and became grouped together in bursts to a greater and greater degree (fig. 3A). These inter-episodal fast waves were identical in appearance with the pre-episodal fast waves and, like the latter, were recorded chiefly from the motor cortex and from certain portions of the cerebellum. The beginning of the typical

episode was signalled by the sudden cessation of the fast waves and the appearance of a short burst of waves at a frequency of 20–30 c.p.s. and a magnitude which varied among the different episodes from 200 to 700 microvolts (fig. 3A). In 0.5 to 2 seconds this initial burst waned, more or less gradually, into a low voltage, somewhat irregular wave pattern. From this minimal level the rhythm progressively increased in magnitude and decreased in frequency (fig. 3, A–L) until a large wave at 2 to 3 c.p.s. was the dominant pattern. The episode was

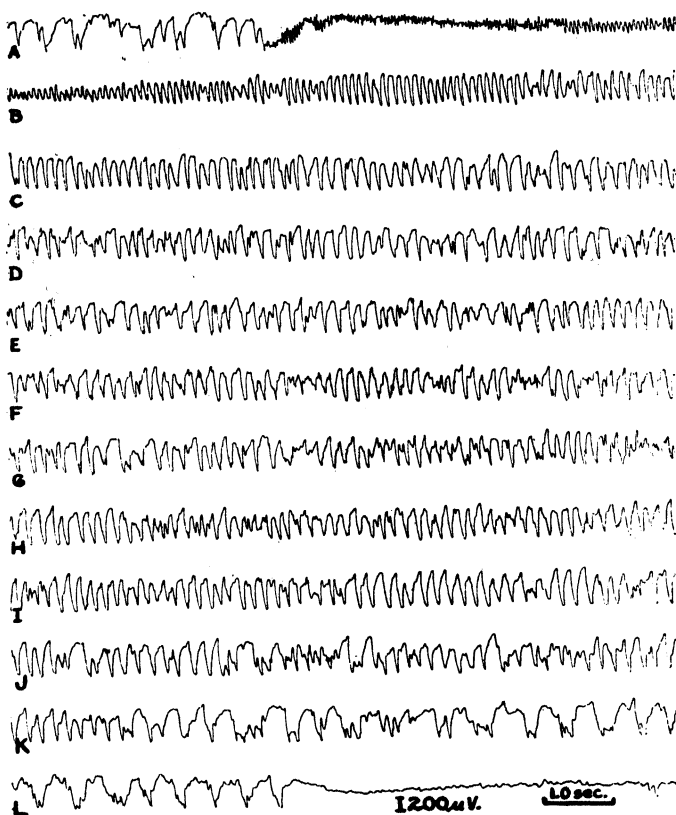


Fig. 3. Continuous records illustrating a complete typical episode recorded from the right motor cortex (area 4 leg) of a monkey. Screw electrodes were employed and the animal received 75 mgm. per kgm. of DDT intravenously.

terminated suddenly by the cessation of the slow waves (fig. 3L) and the establishment of a relatively quiet base line upon which level the next series of fast waves was developed, to initiate, with some variation, the next seizure. There was considerable variation in the seizures from different cortical regions. The discharge was typically best developed at the motor cortex, from which the complete episode shown in figure 3 was recorded.

In cats, as in monkeys, the period immediately following a seizure was one of relatively depressed electrical activity. Upon this background fast waves

appeared and increased in magnitude and frequency. The onset of the typical discharge was characterized by the appearance of a burst of waves at 15-30 c.p.s. The remainder of the episode consisted of a pattern of waves of decreasing frequency and increasing magnitude. The cat cortex, however, was unable to maintain a regular rhythm at as low a frequency as the monkey cortex.

Many variations from these typical seizures were observed, especially in cats. In some cases the initial burst was short-lived or abortive, leading not to a typical episode, but to a continuation of the inter-episodal activity. In such cases several of these abortive bursts sometimes occurred before one of them was followed by the remainder of the episode. Sudden shifts in wave form and frequency occurred in cats during many of the episodes. Although the typical discharge was followed by a period of electrical depression upon which fast waves appeared before the next seizure, a sudden initial burst occurring after the end of an episode sometimes initiated a new discharge without passing through the period of fast waves.

Neither the fast waves nor the episodes appeared to be dependent upon impulses reaching the brain via the spinal cord, for these electrical manifestations of DDT were recorded after complete cord transection in the cat, the transection being at the level of the atlanto-occipital membrane.

Localization and spread in the cat. The fast waves and initial bursts of the cortex of non-anesthetized cats were most prominently developed in the motor cortex or areas close to it. In other cortical regions these electrical manifestations were of minor magnitude. Definite synchronization of the fast waves of the vermal cerebellar lead with fast waves of the motor cortex was also recorded. The cerebellar hemispheres gave no indication of such synchronization. Although the initial bursts and the early phases of the cortical episodes were largely localized to the motor area, the later slow-wave phase of the seizures was able to spread to cortical regions not initially involved. Thus an episode, appearing in the motor cortex, did not remain localized, but, especially during the later stages of the seizure, appeared in other cortical regions and persisted in these even after the discharge in the motor cortex had passed away. It appears, as Adrian (8) and Rosenblueth and Cannon (9) have indicated, that a discharge in a particular cortical region is not dependent for its maintenance on a specific pace-maker, but that all the conditions necessary for the continuance of the seizure are inherent in the region.

Localization and spread in the monkey. Monkeys proved to be very useful in demonstrating the localization of both pre-episodal and inter-episodal fast waves. This localization is summarized in the electrograms of figure 4. The marked development of synchronized, predominantly positive fast waves in areas 4 and 6 is shown in records B-H which were recorded simultaneously. The synchronization of these positive waves with negative waves from the vermis is also indicated (A, C). The absence of significant fast wave activity from the ansiform lobe (G), the visual cortex (E, M₁, N, N₁, R) and area 7 (M, L) is evident. In areas adjacent to the motor cortex (K, S, T, V) the presence of small but definite fast wave activity is obvious. The synchronization of fast wave patterns in the

motor areas with similar activity in the cerebellum was demonstrated, not only by means of screw electrodes in the calvarium, but also by means of pial electrodes. Taken together all the results indicate that the pyramis and lobulus simplex were prominently synchronized with the motor cortex whereas the lobulus ansiformis was the source of no such prominent synchronization.

In the monkey, as in the cat, the later, slow-wave portions of the periodic episodes spread to cortical areas which did not participate in the fast wave and initial burst activity. In the cerebral cortex both the fast wave activity which

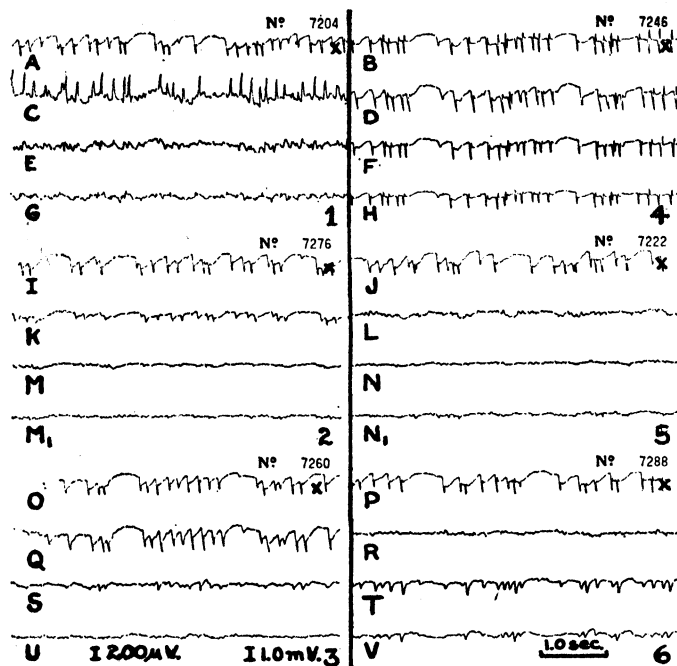


Fig. 4. Electrograms obtained from a monkey by means of screw electrodes showing the localization of the pre-episodal fast waves. Six different recordings (1-6) are shown. Each recording includes the lead from the right area 4 leg marked with an X. The other leads are: vermis (C), area 17 (E, M₁, N, N₁, R), right ansiform lobe (G), area 6 leg (D, Q), area 6 arm (F), area 4 arm (H), area 5 (K), area 7 (M, L, U) and area 9 (S, T, V).

preceded an episode and the initial phases of the episode itself were localized in the same areas. The same relation either does not exist or is doubtful in the case of the cerebellum, for although the typical negative fast waves were recorded from the vermis and not from the ansiform lobes, the initial bursts which introduced the episodes were recorded from both the ansiform and vermician leads, although the burst was more prominent in the latter lead. It should be noted that different episodes as recorded from the cerebellum were more variable than the same episodes led from the motor cortex. Initial bursts were poorly developed or prominent, and slow waves were at times definite, at other times difficult

to identify. This variability made it difficult to study the precise localization and the spread of the episodes over the cerebellar cortex, but it is clear that episodes synchronous with the seizures of the motor cortex took place in the cerebellum.

Interpretation. The obvious alteration in cerebellar activity that followed injection of DDT, in conjunction with the symptomatology of DDT poisoning, which resembles in many characteristics the symptoms of cerebellar deficiency in both the cat (Fulton, Liddell and Rioch; 10) and the monkey (Aring and Fulton, 11), and in conjunction with the observed lesions in the dentate nucleus after DDT (Haymaker and Ginzler, 5) suggests the cerebellum or cerebellar pathways as one possible site of action for the DDT molecule. It is known (Adrian, 12) that the spontaneous electrical rhythm of the cerebellum is increased in magnitude and frequency by afferent impulses and it is possible that the effect of DDT observed with lightly anesthetized animals (fig. 1) was through an excitation of cerebellar afferent pathways. It is important to note that the cerebellar activity reported here was of the same general frequency range as the cortical rhythm. A fast (150–250 c.p.s.) spontaneous activity at a low level (20 microvolts) was recorded from the cerebellum by Adrian (12). Because of the frequency limitation of the ink-writers this fast activity was not recorded in the present experiments.

The typical DDT electrical episode resembles certain electrical seizures recorded from the cerebral cortex which are known to be associated with tonic-clonic motor manifestations. The latter include the grand mal epileptic attacks in humans (Gibbs, Lennox and Gibbs; 13) and the seizures which result from the administration of drugs such as metrazol (Goodwin, Lloyd and Hall; 14; Davis and Sulzbach; 15), camphor (Lennox, Gibbs and Gibbs; 16) and thujone (Adrian and Matthews, 17). All these are characterized by a preliminary period of grouped waves at a relatively low frequency, followed by a burst of relatively high voltage, high frequency and more or less regular rhythm which decreases in frequency, and the discharge ends, more or less suddenly. A striking similarity, even to details, is seen between the DDT-induced episodes and the self-sustained tonic-clonic cortical discharges to electrical stimulation of the cortex (Rosenblueth and Cannon, 9). Thus it appears that irrespective of the agent which provokes the grand mal attack, the basic features of the electrical pattern are identical, indicating that the determining factors in these electrical phenomena are factors of cortical organization and neuronal physiology.

The striking synchronization of the pre-episodal and inter-episodal fast waves between the motor cortex and certain areas of the cerebellum suggests a definite relationship. Either one of these brain areas was firing into the other or both were being simultaneously driven by a group of neurones linked to both. Accurate timing of these waves with a rapidly responding instrument might aid in reaching a decision, but this was not possible in the case of records made with an ink-writer. The polarity of these waves may be of significance. It has already been noted that in the monkey, whereas the cortical fast waves were positive in sign, the corresponding cerebellar deflections were either negative or

else diphasic with an initial negative swing. In his analysis of cortical potentials mediated by way of the corpus callosum, Curtis (18) concluded that surface positive waves indicate corticopetal impulses while surface negative waves are associated with corticofugal activity. On the basis of polarity the cerebellar-cortical synchronization could be explained as being the result of efferent discharges from the cerebellum to the motor cortex. Not all investigators, however, agree with Curtis' interpretation. Dempsey and Morrison (19) and Leão (20) believe that purely surface cortical elements may give rise to surface positive waves.

An examination of the localization of the fast waves in the cerebellum in terms of the known connections of the cerebellum may be of value in evaluating the source of the cortical-cerebellar synchronization. It is clear from the studies of Adrian (12), of Curtis (21) and of Dow (22) that the ansiform folia constitute important receiving areas for impulses from the motor cortex. On the basis of this evidence it is difficult to interpret the fast waves that were recorded from the pyramis and lobulus simplex, and which were absent or insignificant in leads from the ansiform lobe, as representing afferent discharges from the motor cortex. On the basis of known efferent connections of the cortex of the ansiform lobe with the motor cortex (Dow, 23) and in the absence of known efferent connections from the pyramis and lobulus simplex to the motor cortex, it is also difficult to reconcile the present findings with the concept that the fast waves represent discharges from the cerebellum to the motor cortex. We are thus left with the third possibility that both the motor cortex and the cerebellum were being simultaneously activated by impulses from a mass of neurones linked to both areas. In the light of our present knowledge of the action of DDT, any attempt to suggest the site of such activation would be purely speculative.

SUMMARY AND CONCLUSIONS

Following the intravenous administration of DDT emulsions into cats and monkeys which had previously been treated with small doses of sodium pentobarbital to suppress the convulsive action of DDT, the following alterations occurred in the spontaneous electrical potentials recorded from either the exposed or non-exposed cerebral cortex and cerebellum:

1. The cerebellar rhythm progressively increased in magnitude over the course of 1 to 2 hours to a level 2 to 5 times that recorded during the pre-DDT period. The pattern remained essentially the same in form except for a slight increase in frequency.

2. The activity from the cortex increased slightly in magnitude and in frequency, but the chief effect, especially in cats, was the conversion of an electrical pattern, which before DDT consisted of irregular bursts of 8-12 c.p.s. waves, into an almost continuous and regular rhythm at the same frequency or only slightly higher.

In non-anesthetized, curarized cats and monkeys characteristic periodic electrical manifestations were recorded from the cerebral cortex and cerebellum following the administration of DDT. Prior to the initial electrical seizure,

fast waves appeared in the electrograms. Completely synchronized in the motor cortex and cerebellum, these fast waves, in the monkey, were surface positive at the motor cortex and either surface negative or diphasic, with an initial negative deflection, at the cerebellum. The fast waves increased in magnitude and frequency of occurrence until eventually periodic electrical seizures appeared in both the motor cortex and cerebellum. These seizures were in general similar to the electrical pattern in grand mal epilepsy in humans and to the tonic-clonic electrical manifestations following the administration of convulsive drugs or following electrical stimulation of the cortex.

In monkeys the fast waves were most prominent in leads from areas 4 and 6. With leads placed progressively away from the motor cortex, the fast waves became progressively less prominent. In the cerebellum the fast waves were recorded most prominently from the pyramis vermis and portions of the lobulus simplex. In most cases the lobulus ansiformis was silent or relatively inconspicuous with respect to these fast waves. The cerebellum, like the motor cortex, participated in the periodic, tonic-clonic electrical manifestations of DDT. The ansiform lobe as well as the vermis participated in these discharges.

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REFERENCES

- (1) WOODWARD, G., A. A. NELSON AND H. O. CALVERY. *J. Pharmacol. and Exper. Therap.* **82**: 152, 1944.
- (2) SMITH, M. I. AND E. F. STOHLMAN. *Pub. Health Rep.* **59**: 984, 1944.
- (3) BING, R. J., B. McNAMARA AND F. H. HOPKINS. Personal communication.
- (4) PHILIPS, F. S. AND A. GILMAN. Personal communication.
- (5) HAYMAKER, W. AND A. M. GINZLER. Personal communication.
- (6) PICK, E. P. AND K. UNNA. *J. Pharmacol. and Exper. Therap.* **83**: 59, 1945.
- (7) HASSETT, C. Personal communication.
- (8) ADRIAN, E. D. *J. Physiol.* **88**: 127, 1937.
- (9) ROSENBLUETH, A. AND W. B. CANNON. *This Journal* **135**: 690, 1942.
- (10) FULTON, J. F., E. G. T. LIDDELL AND D. McK. RIOCH. *Arch. Neurol. Psychiat.* **28**: 542, 1932.
- (11) ARING, C. D. AND J. F. FULTON. *Arch. Neurol. Psychiat.* **35**: 439, 1936.
- (12) ADRIAN, E. D. *Brain* **66**: 289, 1943.
- (13) GIBBS, F. A., W. G. LENNOX AND E. L. GIBBS. *Arch. Neurol. Psychiat.* **36**: 1225, 1936.
- (14) GOODWIN, J. E., D. P. C. LLOYD AND G. E. HALL. *Proc. Soc. Exper. Biol. Med.* **38**: 897, 1938.
- (15) DAVIS, P. A. AND W. SULZBACH. *Arch. Neurol. Psychiat.* **43**: 341, 1940.
- (16) LENNOX, W. G., F. A. GIBBS AND E. L. GIBBS. *Arch. Neurol. Psychiat.* **36**: 1236, 1936.
- (17) ADRIAN, E. D. AND B. H. C. MATTHEWS. *J. Physiol.* **81**: 440, 1934.
- (18) CURTIS, H. J. *J. Neurophysiol.* **3**: 414, 1940.
- (19) DEMPSEY, E. W. AND R. S. MORISON. *This Journal* **138**: 283, 1943.
- (20) LEÃO, A. A. P. *J. Neurophysiol.* **7**: 359, 1944.
- (21) CURTIS, H. J. *Proc. Soc. Exper. Biol. Med.* **44**: 664, 1940.
- (22) DOW, R. S. *J. Neurophysiol.* **5**: 121, 1942.
- (23) DOW, R. S. *Biol. Rev.* **17**: 179, 1942.

BICARBONATE AND THE RENAL REGULATION OF ACID BASE BALANCE¹

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Under the usual circumstance of ingestion of an acid ash diet the normal individual is faced with a deficit of available base. Those stores of circulating bicarbonate which enter the glomerular filtrate along with other crystalloids of the plasma are carefully conserved; only minute amounts are lost in the urine. But when an alkaline ash diet is ingested or sodium bicarbonate is administered, supplies of available base exceed the needs of the body, and the excess is excreted in the urine as bicarbonate. Reabsorption and excretion of bicarbonate constitute, therefore, the primary renal means of defending the body against depletion and expansion of its alkali reserve. The quantitative importance of the reabsorptive processes in the economy of the human body can be illustrated by a simple calculation. Approximately 190 liters of plasma, containing on an average 25 millimols of bicarbonate per liter, are filtered through the glomeruli in 24 hours (20). Thus, 4,750 millimols of bicarbonate, or 400 grams when expressed as the sodium salt, are tentatively excreted into the tubular urine each day, roughly 5 times the total body store of available base. A little over 2 millimols are excreted per day in 1.5 liters of urine of pH 6.0. Thus the reabsorptive mechanism is 99.95 per cent efficient under ordinary conditions. In contrast, the excretory processes are somewhat less impressive, although more than 100 grams of sodium bicarbonate have been ingested and excreted per day on certain therapeutic and experimental regimes.

The present paper is concerned with two aspects of the renal regulation of acid base balance: 1, a quantitation of the relationship between the plasma concentration and the rates of tubular reabsorption and urinary excretion of bicarbonate; 2, an elucidation of the relationship between the mechanism for the reabsorption of bicarbonate and the mechanism for acidifying the urine.

METHODS. Our experiments have been performed on normal female mongrel dogs trained to lie quietly with loose restraint on a comfortable animal board. The dogs were routinely fasted for 16 to 20 hours before use, and were well hydrated at the start of each experiment by the administration of approximately 40 cc. of water per kilo of body weight by stomach tube. In several experiments plasma bicarbonate was reduced by the oral administration of 200 to 300 cc. of 3 per cent ammonium chloride per day for several days prior to the experiment. In all experiments plasma bicarbonate was increased by the intravenous

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infusion of from 1 to 8 per cent sodium bicarbonate at a rate of 10 cc. per minute. The creatinine clearance was used as a measure of glomerular filtration rate.

Urine was collected with the following special precautions to prevent loss of carbon dioxide. A short silk retention catheter was introduced into the bladder and a rubber catheter of sufficient length to reach the bottom of a 250 cc. graduated cylinder was slipped over its free end. Urine was allowed to drain continuously into the cylinder beneath a heavy layer of paraffin oil. The bladder was emptied at the end of each collection period by manual compression of the lower abdomen; the rubber catheter was disconnected, and its contained urine was added to the specimen. Since urine flows were maintained at rates above 5 cc. per minute, fairly accurate collections were possible, although the bladder was not washed out. The urine specimen was mixed in the cylinder by gently stirring it with a baffle plate attached to a wire which dipped beneath the oil. Samples were withdrawn in pipettes for carbon dioxide content and pH determinations.

Special precautions were likewise taken to prevent loss of carbon dioxide during the collection and centrifugation of arterial blood samples. A retention needle fitted with a tight stylet was introduced into the femoral artery at the start of the experiment. Blood samples were drawn in 20 cc. oiled syringes containing 1 drop of neutral saturated potassium oxalate per 10 cc. of blood. A small amount of mercury served to mix blood and oxalate in the syringe. A portion of the blood was ejected through a spinal needle into a heavy walled centrifuge tube containing oil, the oil was displaced, and the completely filled tube was sealed with a rubber ampoule stopper. The blood was centrifuged immediately and stored in a refrigerator until the supernatant plasma could be analyzed for carbon dioxide (within 3 hrs.). Another portion of the blood was introduced into a 2 cc. Van Slyke stopcock pipette and stored on ice until pH measurement could be made. Since the pH of plasma and whole blood stored as long as 6 hours was identical with that of whole blood measured immediately, centrifuging and storing procedures were deemed adequate. A third portion of the blood was centrifuged without special precautions and plasma filtrates were prepared as previously described for the analysis of creatinine (14).

The carbon dioxide content of plasma and urine was determined by the method of Van Slyke and Neill (21). Analyses were performed on samples of urine varying from 5 cc. for acid urines to 0.2 cc. for the most alkaline urines. Plasma samples ranged from 1.0 to 0.2 cc. The pH of samples of urine and of whole blood was measured without exposure to air in a McInnes type glass electrode using a Cambridge pH meter. Measurements were made at room temperature (usually elevated to 30°C.) and corrected to 37°C. by subtracting 0.01 pH unit per degree difference in temperature (9). Bicarbonate and carbonic acid concentrations were calculated from carbon dioxide content and pH, using the Henderson-Hasselbalch equation and a pK' of 6.1 for both blood and urine. Carbonic acid concentrations were converted to carbon dioxide pressures using $\alpha = 0.0301$ for plasma, and $\alpha = 0.0309$ for urine according to Sendroy et al. (16).

The quantity of bicarbonate filtered through the glomeruli was calculated as the product of the plasma concentration and the glomerular filtration rate (creatinine clearance). The quantity excreted was calculated as the product

of the urine concentration and the urine flow. The difference between the quantity filtered and the quantity excreted is the quantity reabsorbed.

RESULTS. *Reabsorption and excretion of bicarbonate as a function of plasma concentration.* The relationship between the plasma concentration of bicarbonate and the quantity of bicarbonate filtered, excreted, and reabsorbed is illustrated

TABLE 1

Experiments illustrating the relationship between the plasma concentration of bicarbonate and the quantities of bicarbonate filtered, excreted and reabsorbed in the dog. Experiment 1 was performed in the fasting state, experiment 2 after feeding meal.

TOTAL CONCURE- RENT TIME	GLOMER- ULAR FILTRA- TION RATE	ARTERIAL PLASMA CONCENTRATION			URINE FLOW	URINE CONCENTRATION			BICARBONATE					
		pH	Bicar- bonate	CO ₂ pres- sure		pH	Bicar- bonate	CO ₂ pressure	Filtered	Ex- creted	Re- absorbed	per 100 cc. glomerular filtrate		
												Excreted	Reab- sorbed	
Experiment 1; dog 1; fasted														
min.	cc./min.		mM. /l.	mm. Hg	cc. /min.		mM. /l.	mm. Hg	mM. /min.	mM. /min.	mM. /min.	mM.	mM.	
85	Infuse: 0.0% NaHCO ₃ ; 10 cc. per minute													
105-115	73.9	7.25	10.9	25.9	4.4	5.20	0.08	22.0	0.805	0.0004	0.805	0.0005	1.09	
115-125	72.4	7.25	10.2	23.9	8.2	5.19	0.10	26.2	0.738	0.0008	0.737	0.0011	1.02	
126	Infuse: 1.7% NaHCO ₃ ; 10 cc. per minute													
135-145	82.3	7.33	14.8	28.9	9.2	5.79	0.41	27.1	1.22	0.0037	1.22	0.004	1.48	
145-155	78.9	7.41	18.2	29.9	6.7	5.60	0.22	23.3	1.44	0.0015	1.44	0.002	1.82	
156	Infuse: 3.4% NaHCO ₃ ; 10 cc. per minute													
165-175	78.9	7.55	29.4	34.8	11.8	7.59	32.1	33.6	2.32	0.38	1.94	0.48	2.46	
175-185	76.3	7.61	34.4	35.5	13.6	7.72	56.5	44.0	2.62	0.77	1.85	1.01	2.43	
186	Infuse: 6.8% NaHCO ₃ ; 10 cc. per minute													
195-205	81.0	7.69	47.0	40.0	23.9	7.78	82.4	56.0	3.81	1.97	1.84	2.43	2.27	
205-215	77.8	7.73	58.6	45.8	29.2	7.81	94.2	59.5	4.56	2.75	1.81	3.53	2.33	
Experiment 2; dog 1; fed														
40	Infuse: 3.2% NaHCO ₃ ; 10 cc. per minute													
60-70	91.8	7.50	31.7	41.8	14.4	7.43	33.3	50.2	2.91	0.48	2.43	0.52	2.04	
70-80	86.4	7.56	34.4	39.6	15.2	7.57	51.4	56.4	2.98	0.78	2.20	0.90	2.54	
80-90	97.6	7.59	36.1	38.9	11.0	7.76	96.3	68.3	3.53	1.06	2.47	1.09	2.53	
91	Infuse: 4.6% NaHCO ₃ ; 10 cc. per minute													
95-105	100.7	7.64	43.9	42.2	13.2	7.83	142.	85.5	4.42	1.87	2.55	1.86	2.53	
105-115	97.6	7.68	48.9	42.8	14.8	7.87	161.	88.5	4.77	2.38	2.39	2.44	2.45	
115-125	98.2	7.70	51.5	42.8	15.2	7.89	175.	92.0	5.05	2.66	2.39	2.71	2.44	

by experiment 1 in table 1. The low plasma concentration, evident in the first 2 clearance periods of this experiment, resulted from the oral administration of 200 cc. of 3 per cent ammonium chloride per day for 6 days prior to the experiment. Because of the low plasma bicarbonate, the quantity filtered through the glomeruli per unit of time was relatively small, and essentially all (99.95 per cent)

of that filtered was reabsorbed in its passage through the renal tubules. The urine contained negligible quantities of bicarbonate and was highly acid, pH 5.20. As the plasma concentration of bicarbonate was increased in stepwise fashion by the infusion of solutions of sodium bicarbonate of increasing concentration, the quantity filtered through the glomeruli increased in proportion. In the 3rd and 4th clearance periods as in the first two, essentially all the filtered bicarbonate was reabsorbed. However, when the quantity filtered exceeded 2.0 millimols per minute, frank excretion of bicarbonate occurred (periods 5 to 8). A limiting rate of reabsorption amounting to 1.8 to 1.9 millimols per minute was attained, which did not vary appreciably with plasma concentration. All of the bicarbonate filtered, above this limited quantity reabsorbed, was excreted in the urine.

Eighteen experiments similar to the one presented in table 1, comprising a total of 130 clearance periods, were performed on 4 dogs. These dogs varied considerably in functional renal capacity, their creatinine clearances ranging from 46 to 101 cc. per minute. To compare these animals upon a more or less uniform basis, reabsorptive and excretory capacities were calculated in terms of millimols of bicarbonate reabsorbed and excreted per 100 cc. of glomerular filtrate. Such values for experiment 1 are given in the last two columns of table 1. The data collected in all 18 experiments are plotted in figure 1. It is apparent from this figure that when the plasma concentration was below normal (10 to 20 millimols per liter) essentially all of the filtered bicarbonate was reabsorbed, and the quantity excreted was negligible. All animals behaved uniformly in this range of plasma concentration. In contrast, variability in completeness of reabsorption was noted in the different animals within the range of 20 to 25 millimols of bicarbonate per liter of plasma. Above 25 millimols per liter all animals excreted appreciable amounts of bicarbonate and the quantity excreted increased in linear proportion to the increase in plasma concentration. The average reabsorptive capacity above the threshold for frank excretion was 2.5 millimols per 100 cc. of glomerular filtrate. Dog 7 averaged somewhat lower than this mean value, dog 6 somewhat higher.

It is obvious from these data that the renal threshold for frank excretion approximates 25 millimols of bicarbonate per liter of plasma under the conditions of our experiments. Two factors must be considered in making specific applications of this experimental datum. The first factor is polyuria, necessitated in our experiments by our methods of urine collection. Urine pH usually rises moderately as urine flow increases, especially if the initial reaction is acid (1), and this increase in pH is accompanied by some slight elevation in the excretion of bicarbonate. Thus our diuretic animals might be expected to have somewhat lower renal thresholds than those with more usual urine flows. The second and more significant factor is that both the pattern and body store of electrolyte in our experimental animals were essentially normal except for the superimposed alterations in bicarbonate. In pathological states characterized by reduced plasma bicarbonate, it is usual to find a depletion of total body electrolyte, and common to find a disturbance of pattern as well (2, 12). We have observed that alterations in plasma chloride affect bicarbonate reabsorption even though the

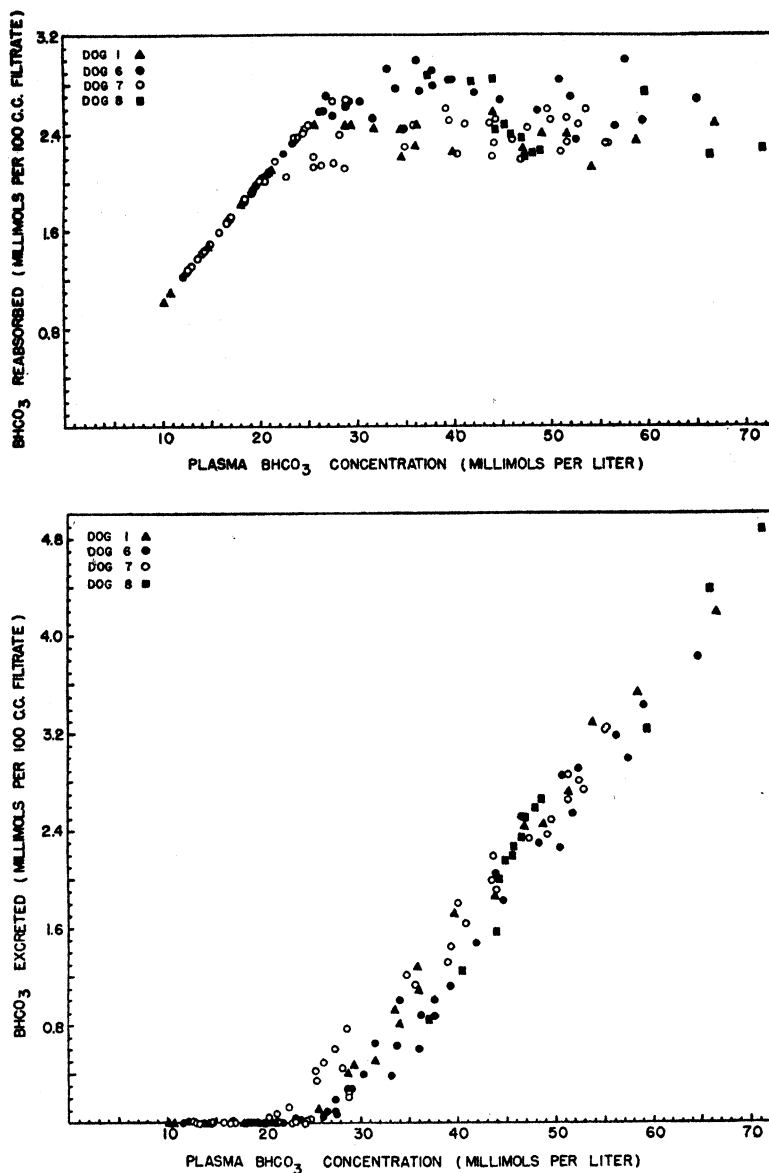


Fig. 1. The renal reabsorption and excretion of bicarbonate in the dog as a function of plasma concentration. Note that the quantities reabsorbed and excreted are expressed in millimols per 100 cc. of glomerular filtrate.

chloride is administered as the sodium salt. When the plasma cation pattern is altered by the administration of potassium chloride, it is probable that even greater changes in reabsorptive capacity occur. Accordingly, in disease states

the renal bicarbonate threshold is subject to wider variations than those we have observed in our experimental animals.

Reabsorption of bicarbonate as a function of glomerular filtration rate. That a correlation exists between glomerular filtration rate and tubular reabsorptive capacity is implied by the data of figure 1. Such a correlation is to be expected in comparing animals of differing renal functional capacities for an obvious morphological correlation exists normally between the extent of the filtering surface of the glomeruli and the reabsorptive mass of the renal tubules. A question of more functional significance is whether or not in a given animal the tubular reab-

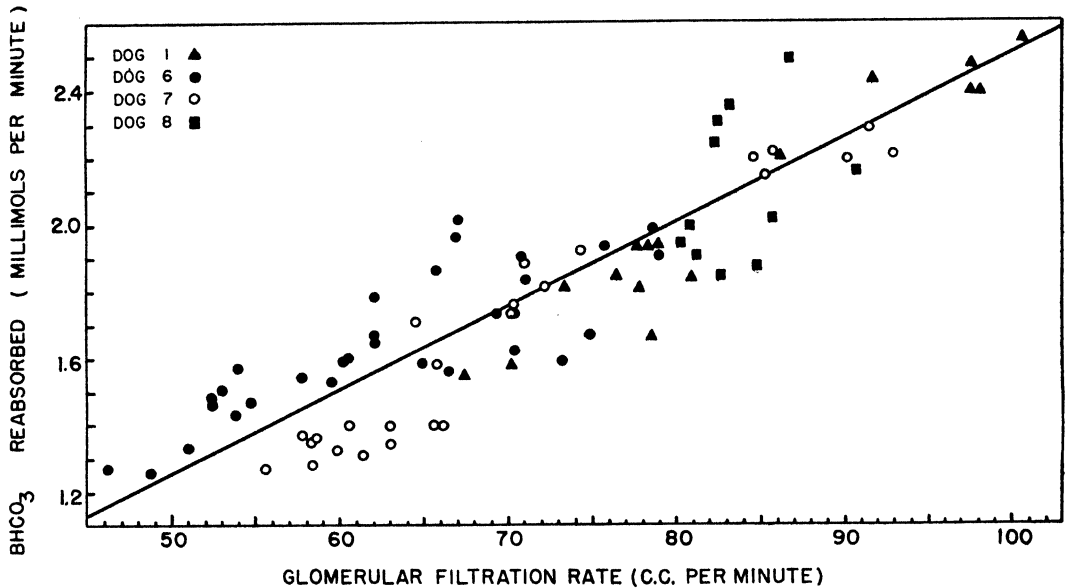


Fig. 2. The renal reabsorption of bicarbonate in the dog as a function of the rate of glomerular filtration. Note that the quantity reabsorbed is expressed in millimols per minute. All reabsorptive capacities were determined at plasma bicarbonate concentrations well above the renal threshold. Filtration rate in a given animal was varied by fasting and by feeding meat.

sorptive capacity for bicarbonate varies with physiologically induced fluctuations in glomerular filtration rate. That reabsorptive capacity does so vary is evident from a comparison of experiments 1 and 2 in table 1, and from a study of the data in figure 2.

Experiment 1 was performed on a fasted animal some 16 to 20 hours after the last meal, whereas experiment 2 was performed on the same animal 3 hours after feeding 2 lbs. of lean chopped meat. Glomerular filtration rate increased from an average of 77.8 cc. per minute in experiment 1 to 95.4 cc. per minute in experiment 2 as a consequence of the feeding of meat. If one compares the last 4 periods of experiment 1, in which the bicarbonate threshold had been exceeded,

with periods at comparable plasma concentrations in experiment 2, it is apparent that the increase in filtration rate was accompanied by an essentially equivalent increase in reabsorptive capacity. Consequently reabsorptive capacity expressed in millimols per 100 cc. of filtrate remained nearly the same.

In figure 2 the quantity of bicarbonate reabsorbed, expressed in millimols per minute, is plotted against the glomerular filtration rate, including all clearance periods from figure 1 in which the renal threshold for gross excretion had been exceeded. The line represents the reabsorption of 2.5 millimols of bicarbonate per 100 cc. of filtrate. From this graph it is evident that there is a direct correlation between the tubular reabsorptive capacity and glomerular filtration rate in animals with differing renal functional capacities. It is likewise evident in any given animal that functional increases in filtration rate are accompanied by essentially equivalent increases in reabsorptive capacity. This latter finding is of significance in any interpretation of the reabsorptive limitation evident in figure 1. In the usual sense of the term, T_m or tubular maximum reabsorptive capacity, cannot be applied to bicarbonate, for the tubular capacity to reabsorb, although limited and independent of plasma concentration at any given filtration rate, is nevertheless a direct function of filtration rate.

Interaction in the reabsorption of chloride and bicarbonate. The plasma concentrations of chloride and bicarbonate are normally maintained at their characteristic levels by the independent excretion of that anionic component which is present in excess. However, it has been repeatedly observed that total plasma anion² concentration tends to be maintained in the face of marked alterations in anion pattern. Thus in alkalosis produced by pernicious vomiting, plasma chloride is low and plasma bicarbonate correspondingly high (8). Conversely in acidosis produced by diarrhea, plasma bicarbonate is low and plasma chloride correspondingly high (7). It is reasonable to infer that the renal thresholds for these two anions are interrelated in some fashion, the effect of which is to maintain the sum of their plasma concentrations within nearly normal limits. Experiments 3 and 4 in table 2 indicate the extent of this interrelationship.

In experiment 3, the initial two clearance periods serve as controls. Plasma bicarbonate and chloride concentrations were within a range of low normal values, and urinary excretion of both bicarbonate and chloride was low. As a consequence of the low bicarbonate excretion, the urine was moderately acid (pH 6.24). The infusion of sodium chloride (3 per cent in periods 3 to 5, 5 per cent in periods 6 to 8 at 10 cc. per min.) progressively increased the plasma chloride concentration from 97 to 150 millimols per liter. Little change occurred in chloride excretion until period 5. At a plasma concentration of 118 millimols per liter a perceptible increase in excretion of chloride occurred, and accompanying this increase was an augmented excretion of bicarbonate and a consequent elevation of urinary pH. Effects are more striking in periods 6 to 8 in which the plasma chloride concentration reached a value of 50 per cent above the initial control value, and in which chloride and bicarbonate excretion rose correspondingly. It is obvious from these data that the increased bicarbonate excretion resulted not

² Perhaps total cation concentration is the true determining factor.

from any increase in the quantity filtered, but from a decrease in the quantity reabsorbed, i.e., from a lowering of the renal threshold.

Experiment 4 reversed the procedure of experiment 3. Elevation of plasma bicarbonate from low initial levels to levels within a range of normal (periods 5

TABLE 2

Experiments illustrating the interrelationships of the renal thresholds for bicarbonate and chloride in the dog.

TOTAL CONCUR- RENT TIME	GLOMER- ULAR FIL- TRA- TION RATE		ARTERIAL PLASMA CON- CENTRATION		URINE FLOW	URINE CONCENTRATION			BICARBONATE			CHLORIDE		
			Bicar- bon- ate	Chlor- ide		pH	Bicar- bonate	Chlor- ide	Filtered	Excreted	Re- absorbed	Filtered	Ex- creted	Reab- sorbed
	Experiment 3; dog 9													
min.	cc./ min.	mM. /l.	mM./l.	cc./ min.		mM./l.	mM./l.	mM. /min.	mM. /min.	mM./ min.	mM./ min.	mM./ min.	mM./ min.	
100	Infuse: 0.0% NaCl; 10 cc. per minute													
120-130	58.8	22.7	95.4	8.1	6.24	1.44	3.00	1.33	0.011	1.32	5.60	0.024	5.58	
130-140	55.8	22.3	97.2	8.4	6.24	1.37	2.60	1.24	0.012	1.23	5.42	0.022	5.40	
141	Infuse: 3.0% NaCl; 10 cc. per minute													
145-155	61.4	21.9	103.	8.0	6.24	1.43	2.40	1.34	0.011	1.33	6.34	0.019	6.32	
155-165	61.8	20.9	111.	7.1	6.31	1.63	3.40	1.29	0.011	1.28	6.88	0.024	6.86	
165-175	63.7	21.0	118.	9.2	6.92	6.75	4.00	1.34	0.062	1.28	7.49	0.037	7.45	
176	Infuse: 5.0% NaCl; 10 cc. per minute													
180-190	57.3	20.0	130.	9.0	7.37	19.7	62.6	1.14	0.176	0.96	7.44	0.563	6.88	
190-200	61.5	19.1	141.	9.3	7.37	20.6	126.	1.17	0.192	0.98	8.64	1.17	7.51	
200-210	57.0	18.4	150.	10.4	7.28	18.0	173.	1.05	0.187	0.86	8.56	1.80	6.76	
Experiment 4; dog 6														
80	Infuse: 0.0% NaHCO ₃ ; 10 cc. per minute													
100-110	56.8	12.7	108.	7.2	6.11	0.92	9.80	0.720	0.007	0.713	6.15	0.071	6.08	
110-120	56.6	12.3	107.	6.7	6.25	1.18	6.40	0.695	0.008	0.687	6.06	0.043	6.02	
121	Infuse: 1.7% NaHCO ₃ ; 10 cc. per minute													
125-135	56.9	14.5	109.	6.7	6.37	1.40	4.80	0.825	0.009	0.816	6.18	0.032	6.15	
135-145	52.9	16.9	108.	7.2	6.37	1.53	6.00	0.895	0.011	0.884	5.71	0.043	5.67	
146	Infuse: 3.4% NaHCO ₃ ; 10 cc. per minute													
150-160	53.6	23.5	105.	8.6	6.40	1.86	7.00	1.26	0.016	1.24	5.64	0.060	5.58	
160-170	54.7	27.6	103.	10.0	6.65	3.70	4.00	1.51	0.037	1.47	5.64	0.040	5.60	
171	Infuse: 6.8% NaHCO ₃ ; 10 cc. per minute													
175-185	62.1	37.7	103.	15.3	7.46	33.5	28.4	2.34	0.510	1.83	6.41	0.435	5.97	
185-195	57.8	44.7	98.2	14.9	7.71	70.3	40.4	2.59	1.05	1.54	5.67	0.736	4.93	

and 6) was without effect on the excretion of either bicarbonate or chloride. However, at high plasma bicarbonate levels (periods 7 and 8), increased excretion of bicarbonate was accompanied by decreased reabsorption and increased excretion of chloride. It is apparent from these experiments that the capacity of the renal tubules to reabsorb bicarbonate is reduced by presenting to them simultaneously an excess of chloride. Similarly their capacity to reabsorb chloride is

reduced by presenting to them an excess of bicarbonate. Some of the variability in renal threshold and in bicarbonate reabsorptive capacity evident in the data of figures 1 and 2 may have been related to variations in plasma chloride concentration.

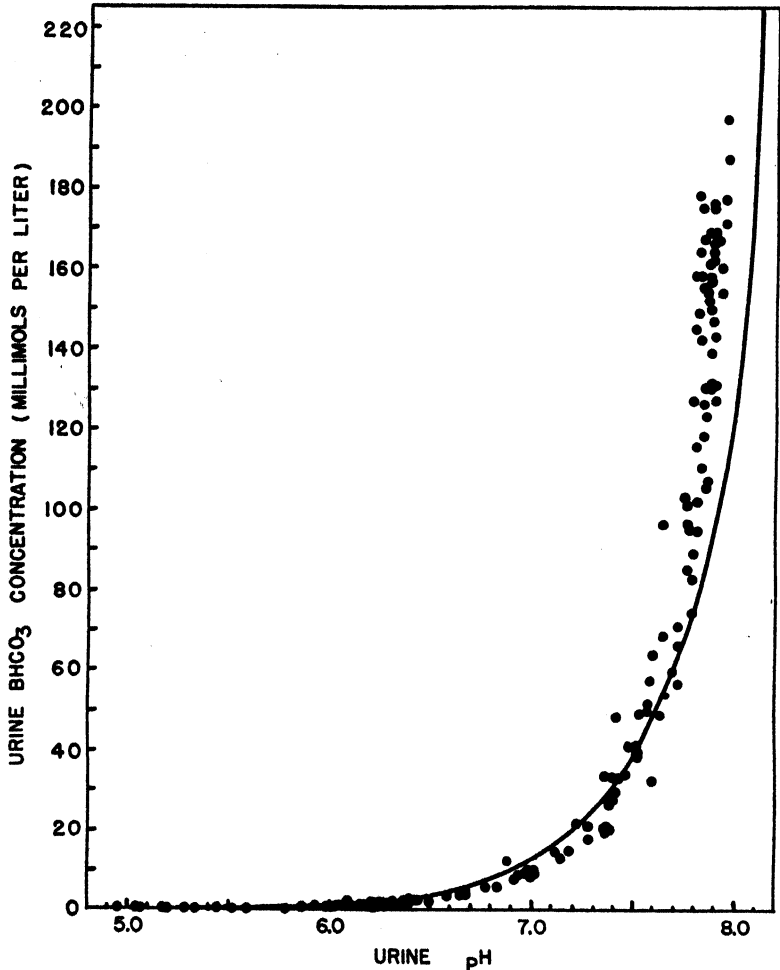


Fig. 3. The relationship between bicarbonate concentration and pH in 160 specimens of dog urine so collected as to prevent the loss of carbon dioxide. The smooth curve was calculated from the Henderson-Hasselbalch equation assuming a constant $p\text{CO}_2$ of 50 mm. Hg.

Bicarbonate excretion and acid-base equilibria in urine. Marshall (10) and Gamble (6) demonstrated that the concentration of bicarbonate in urines more acid than pH 6.0 is very low, and that it rises progressively in urines of increasing alkalinity, especially in those of pH 7.00 or above. They likewise demonstrated that excess body base is excreted almost entirely in the form of bicarbonate, which may increase to a value as high as 220 millimols per liter of urine. Since the

carbon dioxide pressure of the urine roughly approximates that of the blood (5), urines collected so as to avoid loss of carbon dioxide are never more alkaline than pH 8.0. Thus large quantities of base can be eliminated in urine which is only slightly more alkaline than blood.

In figure 3 the bicarbonate concentrations of 160 urine samples collected in 24 experiments are plotted against urine pH. The lowest urine pH was 4.96, the highest 7.96. The lowest bicarbonate concentration was 0.08 millimol per liter, the highest 197 millimols per liter. Between these extremes the relationship between bicarbonate concentration and pH was a uniform regular one and approximated that demanded by the Henderson-Hasselbach equation, assuming a constant carbon dioxide pressure of 50 mm. Hg (cf. smooth curve). However, the deviations from this theoretical curve are significant. Below pH 7.4 an assumed pressure of carbon dioxide of 50 mm. Hg is too high; above pH 7.6 it is too low. Thus the finding by Gamble (5) that urine $p\text{CO}_2$ is relatively constant over a wide range of urine pH is not borne out by our data. One obvious cause for this discrepancy is the very wide range of plasma bicarbonate concentration in our studies, 10 to 70 millimols per liter, which far exceeded the range in Gamble's human subjects. With such a range in plasma bicarbonate one would predict a wide range in plasma $p\text{CO}_2$, and if urine $p\text{CO}_2$ were related to plasma $p\text{CO}_2$, a similar range in urine $p\text{CO}_2$ would be expected.

In figure 4 the $p\text{CO}_2$ of the urine is plotted against the $p\text{CO}_2$ of the plasma, collected simultaneously. Urine $p\text{CO}_2$ varied from 22 mm. Hg to 109 mm. Hg, whereas plasma $p\text{CO}_2$ varied from 25 mm. Hg to 64 mm. Hg. Below a plasma $p\text{CO}_2$ of 40 mm. Hg there was relatively an even distribution of urine values above and below corresponding plasma values. Above a plasma $p\text{CO}_2$ of 40 mm. Hg, urine values tended to exceed plasma values. We fully recognize that our figures for $p\text{CO}_2$, since they are calculated, are subject to a number of sources of error such as variability of pK' and α factors in different samples of blood and urine, random analytical errors, and the possibility of loss of small amounts of carbon dioxide in some samples. However, the following trends seem evident. 1. In acid urines, formed when plasma bicarbonate is subnormal, the urine $p\text{CO}_2$ tends to approximate the arterial plasma $p\text{CO}_2$. Carbon dioxide diffuses rapidly through most tissues, presumably through the renal epithelium as well. One would therefore predict that, within limits, equilibrium would be established between tubular urine, tubular cells, and renal venous blood with respect to carbon dioxide pressure. Since our plasma values are arterial this could mean that in acidosis the renal venous $p\text{CO}_2$ is essentially the same as the arterial $p\text{CO}_2$, i.e., the majority of the carbon dioxide produced in the oxidative metabolism of the kidney is added to the renal venous blood not as carbon dioxide, but as bicarbonate (cf. Pitts and Alexander (13, 15)). 2. On the other hand, in alkaline urines formed when plasma bicarbonate is above normal, the urine $p\text{CO}_2$ exceeds the arterial $p\text{CO}_2$ by a considerable margin and no doubt exceeds the renal venous $p\text{CO}_2$. This could well mean that a part of the reabsorption of bicarbonate occurs in that segment of the renal tubule which acidifies the urine by the exchange of intracellular H^+ ion for Na^+ ion of buffer salts present in the tubular urine (15).

Such an exchange involving bicarbonate would lead to the formation of carbonic acid in the tubular urine. Since the tubular urine contains no carbonic anhydrase, dehydration of carbonic acid to carbon dioxide and diffusion of carbon dioxide across the tubular epithelium into the renal venous blood might be suffi-

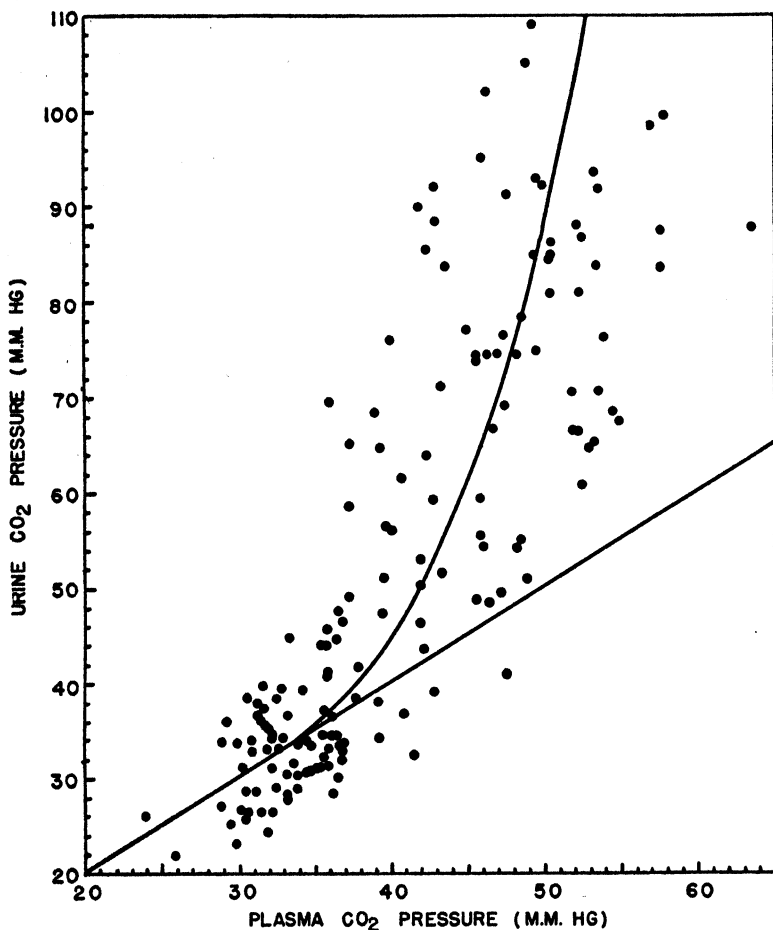


Fig. 4. The relationship between the partial pressure of carbon dioxide in plasma and in urine collected simultaneously. The diagonal straight line indicates equivalence of partial pressure. The curve is the average of all points, fitted by inspection.

ciently delayed to permit a significant elevation in urine $p\text{CO}_2$. Data presented below bear out this supposition.

The distal tubular reabsorption of bicarbonate and the mechanism for acidifying the urine. From a synthesis of the evidence obtained in the amphibian¹ and mammalian kidney we infer that bicarbonate is reabsorbed by two dissimilar renal mechanisms located respectively in the proximal and distal tubules. In the frog and necturus the major part of the filtered bicarbonate is reabsorbed in the

proximal segment of the renal tubules (22). Since the pH of the tubular urine at the end of the proximal segment is identical with that of the original filtrate, this reabsorptive process is an isohydric one (11), i.e., bicarbonate, carbon dioxide, base and water are reabsorbed in the same proportions in which they exist in the original filtrate. The remainder of the bicarbonate is ordinarily reabsorbed in the distal segment of the renal tubules, the process reaching completion at that level at which the urine attains its maximum acidity (11). It was suggested above that this final anisohydric reabsorption of bicarbonate is accomplished by that selfsame mechanism which, by exchanging H^+ ions for Na^+ ions of urinary buffer salts, acidifies the urine (13, 15). Two lines of experimental evidence support this view.

TABLE 3

Experiment illustrating the depression of the reabsorption of bicarbonate which is produced by the administration of sulfanilamide in the dog

TOTAL CONCURRENT TIME	GLOMERULAR FILTRATION RATE	ARTERIAL PLASMA CONCENTRATION				URINE FLOW	URINE CONCENTRATION			BICARBONATE			CHLORIDE		
		pH	Bi-carbonate	Chloride	Sulfanilamide		pH	Bi-carbonate	Chloride	Filtered	Excreted	Reabsorbed	Filtered	Excreted	Reabsorbed

Experiment 5; dog 9															
min.	cc./min.	mM./l.	mM./l.	mM./l.	cc./min.	mM./l.	mM./l.	mM./min.	mM./min.	mM./min.	mM./min.	mM./min.	mM./min.	mM./min.	mM./min.
90	Infuse: 0.0% sulfanilamide; 10 cc. per minute					7.5	6.28	1.43	4.4	1.21	0.011	1.20	5.34	0.033	5.31
115-125	52.9	7.44	22.8	101	0.0	8.0	6.28	1.42	2.8	1.22	0.011	1.21	5.63	0.022	5.61
125-135	55.6	7.44	22.0	101	0.0	7.6	6.38	1.73	1.4	1.19	0.013	1.18	5.45	0.011	5.44
135-145	54.2	7.44	22.0	101	0.0										
146	Prime: 200 cc. 1.5% sulfanilamide														
150	Infuse: 1.2% sulfanilamide; 10 cc. per minute					11.6	7.00	8.25	2.8	1.14	0.096	1.04	5.39	0.031	5.36
155-165	54.1	7.44	21.0	99.6	25.8	11.4	7.00	8.67	3.4	1.16	0.099	1.06	5.54	0.039	5.50
165-175	55.2	7.44	21.0	100	32.6	11.0	7.01	9.35	2.4	1.15	0.103	1.05	5.58	0.026	5.55
175-185	55.2	7.44	20.8	101	39.4	10.5	7.01	9.17	2.2	1.15	0.097	1.05	5.62	0.023	5.60
185-195	55.5	7.44	20.7	101	46.1	10.0	7.00	9.77	2.4	1.11	0.098	1.01	5.47	0.024	5.45
195-205	54.3	7.43	20.4	101	52.8										

When sulfanilamide is administered to an animal in acidosis whose renal mechanism for excretion of titratable acid has been loaded by the infusion of phosphate, the capacity of the kidney to eliminate acid is reduced (13, 15). As is evident from experiment 5 in table 3, the administration of sulfanilamide to a normal animal reduces the capacity of the kidney to reabsorb bicarbonate and causes the excretion of bicarbonate and the formation of an alkaline urine. In other experiments in which bicarbonate was administered at rates sufficient to saturate the reabsorptive mechanism, sulfanilamide likewise depressed reabsorptive capacity. However, the extent of the depression in reabsorptive capacity is small in either circumstance, as would be expected if the majority of the bicarbonate were reabsorbed by a non-sulfanilamide-sensitive proximal tubular mechanism. We infer that the extra moiety of bicarbonate excreted after sulfa-

nilamide is that which is normally reabsorbed by a process of distal tubular exchange of H^+ ions for Na^+ ions. Undoubtedly blockage of this mechanism is incomplete at the plasma concentrations of sulfanilamide attained in this experiment (4, 15). It is worthy of note that sulfanilamide did not effect the reabsorption or excretion of chloride, a point of significance to which we shall return later.

Another line of evidence implicates the mechanism of exchange of H^+ ion for Na^+ ion in the distal tubular reabsorption of bicarbonate. In experiment 6 of table 4 sodium phosphate was infused into an acidotic animal at such a rate as to maintain the plasma concentration of phosphate at a value some 5 times the normal throughout the course of the experiment. In the initial two control periods titratable acid³ was excreted at an average rate of 0.303 milliequivalent per minute. The plasma bicarbonate was then elevated gradually from its initial

TABLE 4

Experiment illustrating the depression of the excretion of titratable acid which is produced by the renal reabsorption of progressively increasing quantities of bicarbonate in the dog

TOTAL CONCURRENT TIME	GLOMER- ULAR FILTRA- TION RATE	ARTERIAL PLASMA CON- CENTRATION			URINE		URINARY EXCRETION RATE			BICARBONATE	
		pH	Bicar- bonate	Phos- phate	Flow	pH	Creati- nine	Phos- phate	Titra- table acid	Reab- sorbed	Ex- creted
Experiment 6; dog 7											
min.	cc./min.		mM./l.	mM./l.	cc./min.		mM/ min.	mM./ min.	mEq./ min.	mM./ min.	mM./ min.
75	Infuse: 0.04 M PO ₄ ; 10 cc. per minute										
100-110	73.2	7.25	12.2	5.48	9.2	4.98	0.188	0.270	0.292	0.89	0.0005
110-120	74.9	7.25	12.2	5.51	8.9	4.94	0.200	0.291	0.314 0.303	0.91	0.0004
121	Infuse: 0.04 M PO ₄ , 0.22 M NaHCO ₃ ; 10 cc. per minute										
130-140	77.4	7.32	15.9	5.40	8.4	5.32	0.215	0.316	0.296	1.23	0.001
140-150	85.2	7.38	18.2	5.29	7.7	5.78	0.225	0.331	0.261	1.55	0.005
150-161	81.8	7.44	21.0	5.29	7.43	6.27	0.216	0.318	0.196	1.69	0.03
161-170	88.3	7.46	22.9	5.44	9.27	6.63	0.248	0.369	0.158	1.91	0.11
170-180	86.6	7.46	24.3	5.18	7.8	6.82	0.235	0.342	0.107	1.96	0.15
180-190	76.2	7.49	25.9	4.95	5.9	6.96	0.203	0.292	0.071	1.80	0.17

low value to a value well within the range of normal by the infusion of sodium bicarbonate. The titratable acidity of the urine diminished and the pH of the urine rose as the quantity of filtered bicarbonate increased. Yet throughout periods 3 to 5 reabsorption of bicarbonate was nearly complete and little appeared in the urine. Only in periods 6 to 8 was excretion of bicarbonate significant. It is evident that the reabsorption of increasing quantities of bicarbonate in periods 3 to 5, unaccompanied by any increase in excretion, in some way diminished the quantity of titratable acid eliminated. We infer that both bicarbonate and phosphate serve as sources of Na^+ ions which are exchanged for H^+ ions across the tubular epithelium. If little bicarbonate and much phosphate are present in

³ The titratable acid figures are calculated from the pH of the urine and the excretion rate of creatinine and phosphate. Calculated and experimentally determined values agree within ± 5 per cent.

the tubular lumen at the site of the urinary acidification, large quantities of secondary phosphate are transformed into the primary form and the titratable acidity of the urine is high. Such conditions are to be expected in acidosis. On the other hand, if much bicarbonate is present in the tubular urine, a part is transformed into carbonic acid, relatively less of the phosphate is changed, and the titratable acidity of the urine is correspondingly reduced. Such conditions are to be expected in alkalosis.

DISCUSSION. Three characteristics of the renal tubular mechanism for the reabsorption of bicarbonate admirably fit it for its function of stabilizing the alkali reserve of the plasma. 1. The mechanism is remarkably efficient in reabsorbing all but a trace of bicarbonate from the tubular urine when the plasma concentration is below normal. Thus none of the depleted body store is wasted. 2. The mechanism is capable of reabsorbing on an average 2.5 millimols of bicarbonate from each 100 cc. of glomerular filtrate. When more than this quantity is delivered into the glomerular filtrate as a consequence of an increase in plasma concentration, the excess is excreted in the urine. Accordingly the plasma bicarbonate concentration tends to stabilize at 2.5 millimols per 100 cc., or as usually expressed, at 25 millimols per liter, when adequate supplies of base are available to the body. 3. The quantity of bicarbonate reabsorbed per unit of time varies directly and nearly proportionally with changes in the rate of glomerular filtration. Consequently the renal bicarbonate threshold is relatively stable and nearly independent of the glomerular filtration rate.

This latter characteristic sets the renal mechanism for reabsorption of bicarbonate apart from those for reabsorption of glucose (18), vitamin C (19), and phosphate (14). The reabsorptive capacities of these latter mechanisms are fixed and independent of both glomerular filtration rate and plasma concentration (above the renal threshold). It has been generally assumed that this type of reabsorptive limitation derives from the presence within the renal tubular cells of a fixed quantity of some cellular component with which the reabsorbed material combines in the process of tubular transfer (17). The rate of breakdown of this tubular complex presumably limits the overall rate of reabsorption. Such a simple explanation obviously will not account for the reabsorption of bicarbonate. Although the quantity of bicarbonate reabsorbed per unit of time is independent of plasma concentration above the renal threshold, it varies directly with the filtration rate.

In attempting to visualize a means by which reabsorptive capacity could be correlated with filtration rate we have arrived at the following modification of Cushny's concept of the tubular reabsorption of fluid of constant composition (3). It is generally assumed that four-fifths of the water filtered through the glomeruli is reabsorbed in its passage through the proximal tubules by an obligatory process (20). If this were true at all rates of glomerular filtration, and if the concentration of bicarbonate in this reabsorbate were limited to a value of not more than 2.5 millimols per 100 cc., the quantity of bicarbonate reabsorbed in the proximal tubule per unit of time would vary in proportion to the glomerular filtration rate. How the concentration of bicarbonate in the proximal reab-

sorbate is limited to 2.5 millimols per 100 cc. is beyond our present comprehension of the problem. However, as a consequence of the operation of such a mechanism up to 2.0 millimols of bicarbonate could be reabsorbed in the proximal tubule from each 100 cc. of glomerular filtrate, i.e., 80 cc. of fluid containing 2.5 millimols per 100 cc. At normal plasma concentrations, roughly 0.5 millimol of bicarbonate would be delivered into the distal tubule for each 100 cc. of original filtrate. If we assume that the distal tubule can reabsorb quantities of this order of magnitude, then the urine will be essentially free of bicarbonate. When more than 0.5 millimol reaches the distal tubule, the excess is excreted. Since proximal reabsorption is quantitatively much greater than distal reabsorption, total bicarbonate reabsorption would vary in rough proportion to glomerular filtration rate no matter what the characteristics of the distal mechanism might be.

There is reason for believing that the distal reabsorptive mechanism differs from the proximal mechanism in at least two ways. 1. Distal reabsorption of bicarbonate is nearly, although not completely, independent of water reabsorption, whereas proximal reabsorption obviously is proportional to water reabsorption. The independence of distal reabsorption of water and of bicarbonate derives from the fact that in man urine flow can be varied over a wide range with only small changes in urine pH and hence only small changes in bicarbonate excretion (1). Since variations in urine flow are effected largely through inverse variations in the quantity of water reabsorbed in the distal tubule (20), it is evident that water and bicarbonate reabsorption are relatively independent. 2. Distal reabsorption of bicarbonate is an anisohydric process, whereas proximal reabsorption is probably an isohydric process. Evidence presented in this paper supports the view that bicarbonate reabsorption is effected indirectly in the distal tubule by the exchange of H^+ ions for Na^+ ions, thereby converting bicarbonate in the tubular urine to carbonic acid. This carbonic acid on dehydration to carbon dioxide diffuses across the tubular epithelium into the renal venous blood. Failure to establish equilibrium across the tubular epithelium would account for the fact that the pCO_2 of urine is often higher than that of blood, especially when the distal mechanism is loaded and bicarbonate appears in the urine.

We have no direct evidence as to the nature of the proximal reabsorptive mechanism in the dog. Reabsorption in this segment of the amphibian kidney occurs isohydrically (11), and we presume the same to be true of the mammalian kidney within limits. However, experiments 3 and 4 indicate that there is a mutual interference in the reabsorption of bicarbonate and chloride when either anion is present in the tubular urine in large excess, and experiment 5 suggests that the site of this interaction is in the proximal, not the distal, tubule. Sulfanilamide, which presumably depresses the distal reabsorption of bicarbonate, has no effect on chloride reabsorption. From this we conclude that the distal chloride and distal bicarbonate reabsorptive mechanisms are independent, and that interaction must occur in the proximal tubule.

SUMMARY

The renal tubular reabsorption of bicarbonate in the normal dog has been assessed at plasma concentrations ranging from 10 to 70 millimols per liter.

1. Under the conditions of our experiments the renal threshold for gross excretion of bicarbonate is approximately 25 millimols per liter of plasma. Below the renal threshold essentially all of the filtered bicarbonate is reabsorbed. Above the renal threshold the rate of excretion of bicarbonate is a linear function of the plasma concentration.

2. On an average the renal tubules reabsorb 2.5 millimols of bicarbonate from each 100 cc. of filtrate when the plasma concentration is above the threshold. The excess filtered, over and above this limited quantity reabsorbed, is excreted in the urine.

3. Although the capacity of the tubules to reabsorb bicarbonate is independent of plasma concentration above the threshold, it is not fixed in the ordinary sense of T_m . Functional increases in filtration rate are accompanied by essentially equivalent increases in tubular reabsorptive capacity. Thus the quantity reabsorbed per 100 cc. of filtrate remains the same, and the renal threshold is independent of filtration rate.

4. The renal thresholds for chloride and bicarbonate are interrelated in such a fashion as to maintain constant the sum of the plasma concentrations of these two anions. Thus an increase in plasma chloride concentration reduces the renal threshold for bicarbonate, and conversely an increase in plasma bicarbonate concentration reduces the renal threshold for chloride.

5. The partial pressure of carbon dioxide in acid urines approximates that of the arterial plasma. In alkaline urines it exceeds that of the plasma by a considerable margin, attaining a maximum observed value of 109 mm. Hg. Under the conditions of our experiments acid urines contain a minimum of 0.08 millimol of bicarbonate per liter, whereas alkaline urines contain a maximum of 197 millimols per liter. Urine pH accordingly varies within limits of 4.96 and 7.96.

6. In acidosis the rate of excretion of titratable acid varies inversely with the quantity of bicarbonate reabsorbed by the renal tubules, even though the rate of excretion of bicarbonate is low and remains essentially unchanged. The administration of large amounts of sulfanilamide not only reduces the rate of excretion of titratable acid but reduces the capacity of the renal tubules to reabsorb bicarbonate. We interpret these two observations as indicating that bicarbonate is reabsorbed in small part by that distal tubular mechanism which is responsible for the elimination of titratable acid.

7. We infer from our data that a much larger moiety of bicarbonate is reabsorbed by an independent proximal tubular mechanism, the characteristics of which largely determine the overall nature of bicarbonate reabsorption as outlined in sections 1 to 4 above.

REFERENCES

- (1) BARCLAY, J. A. AND M. E. NUTT. *J. Physiol.* **103**: 20P, 1945.
- (2) BULGER, H. A., J. P. PETERS, A. J. EISENMAN AND C. LEE. *J. Clin. Investigation* **2**: 213, 1926.
- (3) CUSHNY, A. R. *The secretion of the urine.* Longmans Green & Co., New York, 1917.
- (4) DAVENPORT, H. *J. Biol. Chem.* **158**: 567, 1945.
- (5) GAMBLE, J. L. *Chemical anatomy, physiology and pathology of extracellular fluid.* Harvard Medical School, 1942.

- (6) GAMBLE, J. L. J. Biol. Chem. **51**: 295, 1922.
- (7) HAMILTON, B., L. KAJDI AND D. MEEKER. Am. J. Dis. Child. **38**: 314, 1929.
- (8) HASTINGS, A. B., C. D. MURRAY AND H. A. MURRAY, JR. J. Biol. Chem. **46**: 223, 1921.
- (9) Laboratory manual of field methods for biological assessment of metabolic and nutritional conditions. Harvard Fatigue Laboratory, Boston, 1945.
- (10) MARSHALL, E. K., JR. J. Biol. Chem. **51**: 3, 1922.
- (11) MONTGOMERY, H. AND J. A. PIERCE. This Journal **118**: 144, 1937.
- (12) PETERS, J. P., H. A. BULGER, A. J. EISENMAN AND C. LEE. J. Clin. Investigation **2**: 167, 1925.
- (13) PITTS, R. F. Science **102**: 49, 81, 1945.
- (14) PITTS, R. F. AND R. S. ALEXANDER. This Journal **142**: 648, 1944.
- (15) PITTS, R. F. AND R. S. ALEXANDER. This Journal **144**: 239, 1945.
- (16) SENDROY, J., JR., S. SEELIG AND D. D. VAN SLYKE. J. Biol. Chem. **106**: 463, 1934.
- (17) SHANNON, J. A. Physiol. Rev. **19**: 63, 1939.
- (18) SHANNON, J. A. AND S. FISHER. This Journal **122**: 765, 1938.
- (19) SHERRY, S., G. J. FRIEDMAN, K. PALEY, J. BERKMAN AND E. P. RALLI. This Journal **130**: 276, 1940.
- (20) SMITH, H. W. The physiology of the kidney. Oxford Univ. Press, New York, 1937.
- (21) VAN SLYKE, D. D. AND J. M. NEILL. J. Biol. Chem. **61**: 523, 1924.
- (22) WALKER, A. M., C. L. HUDSON, T. FINDLEY, JR. AND A. N. RICHARDS. This Journal **118**: 121, 1937.

THE STANDARDIZATION OF HEMORRHAGIC SHOCK IN THE RAT: OBSERVATIONS ON THE EFFECTS OF TRANSFUSIONS OF WHOLE BLOOD AND SOME BLOOD SUBSTITUTES¹

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Earlier work in this laboratory has shown that the response of rats to a standard bleeding procedure (removal of about 3 per cent of the body weight of blood in the course of an hour) is rather variable and difficult to reproduce in successive groups of animals. For some experimental purposes this is not a serious difficulty, since it may be desirable to make comparable observations on animals in mild, moderate, or severe states of shock after acute blood loss. In the assay of blood substitutes, however, a confident prediction of the outcome of any bleeding procedure applied to groups of animals is essential if the estimates of effectiveness of therapeutic agents are to have any meaning. A study has therefore been made of the factors influencing the response of rats to hemorrhage and to transfusion. It has been possible to establish conditions for the quantitative estimation of the effectiveness of blood substitutes in rats in severe states of shock: 1, in imminent collapse shortly after acute blood loss, and 2, in a late stage of prolonged severe hemorrhagic shock.

MATERIALS AND METHODS. Male rats of the Sprague-Dawley strain were used throughout. They were fed a standard diet, and were maintained at a constant environmental temperature for at least seven days before being used for experiment. In all instances the rats were fasted for 24 hours before use. During this time they had access to water. The environmental temperature was maintained at $84 \pm 1^\circ\text{F}$. during both the fasting and the experimental periods.

Bleeding procedure. No anesthetic was used. The rats were suspended in canvas slings (1) in which they remained without additional food or water until the end of the observation period, 24 hours after the initiation of a therapeutic measure. Animals alive at this time were counted as survivors. About 2 ml. of blood per 100 sq. cm. of body surface (table 1 footnote), was drawn from the cut end of the tail in the course of one hour. One-sixth of the calculated amount was withdrawn at the beginning of five successive ten-minute intervals, but in the last 10-minute period bleeding was continued until the flow of blood abruptly slowed and the animal no longer responded to a normally painful stimulus. This, rather than the withdrawal of the calculated volume of blood, was regarded as the end-

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point of bleeding. In these circumstances all of the rats die within an hour, most of them within 40 minutes, after the end of bleeding. The term "bleeding volume" will be used hereafter to denote the volume of blood which, when removed as described, leads to death. It is approximately 2 ml. per 100 sq. cm. of body surface.

The condition of the animals can be judged best by the sequence of respiratory events during and after bleeding. Before or shortly after the end of bleeding, the respiratory rate increases to 150 to 200 cycles per minute. This rate is maintained until shortly before death, when the following alterations in respiratory activity take place. 1. An abrupt slowing of respiration to about 60 cycles per minute, accompanied by a marked decrease in depth of respiration, occurs about 5 to 7 minutes before death, and lasts for 2 to 3 minutes. 2. The mouth begins to open with each inspiration. This stage develops in about 20 seconds into stage 3, of forcible gasping inspirations. Within a minute of the onset of gasping,

TABLE 1
Relation between bleeding volume and rate of bleeding

TIME TAKEN TO REMOVE BLOOD	NUMBER OF RATS	BLEEDING VOLUME (ML. PER 100 SQ. CM. OF BODY SURFACE*)
<i>hours</i>		
1-3	14	1.90 (1.57-2.20)
3-5	15	2.09 (1.64-2.47)
5-7	9	2.45 (1.90-2.80)
7-10	7	2.68 (2.44-3.05)
10-15	9	3.11 (2.83-3.42)
19-20	4	3.51 (3.01-3.87)

* Body surface (S) = $KW^{0.60}$, where S = surface area in sq. cm.; K = 12.54; W = fasting body weight in grams (2).

These values of the bleeding volume are not comparable with those of table 2, since they were made before all the factors influencing bleeding volume had been standardized.

the corneal reflex usually disappears, and with another minute respiration usually ceases.

Transfusion procedure. Just after bleeding was ended the left superficial vein of the tail was exposed for about an inch of its course midway between the base and the tip. Heparinized donor blood, or the chosen blood substitute in any instance, was infused into this vein through a no. 26 hypodermic needle. The needle was introduced during stage 1 of the terminal respiratory events, and transfusion was begun in the interval between stages 2 and 3, at rates of 0.2 ml. per 2 seconds for the first ml., 0.2 ml. per 4 seconds for the second ml., then 0.2 ml. per 8 seconds until the volume of blood returned equalled the volume removed to produce the collapse, and 0.2 ml. per 12 seconds for amounts in excess of this volume. In 3 per cent of the rats the onset of stages 2 and 3 was not readily recognizable, and these animals were therefore discarded.

RESULTS. 1. *Factors Affecting the Response of the Rat to Acute Loss of Blood.*
a. Rate of bleeding. Table 1 illustrates the relation between bleeding volume and

the time taken to remove the blood. It is obvious that a fixed bleeding time must be chosen in establishing any standard procedure. For convenience, a fixed bleeding time of one hour was therefore selected. *b. Body size.* The relation of the observed bleeding volumes in a large series of rats, weighing from 96 to 279 grams (fasted) to body weight and to body surface is shown in table 2. Bleeding volume is a linear function of body surface rather than of body weight. This relation has not previously been recognized, but it may have been expected, since total blood volume both in the rat (3) and in man (4) is also a linear function of body surface. A more uniform response of groups of rats to bleeding may be

TABLE 2

Relation between bleeding volume, body weight, and body surface in the male rat

RANGE IN FASTED BODY WEIGHT	NUMBER OF RATS	BLEEDING VOLUME VOLUME OF BLOOD REMOVED IN ONE HOUR WHICH LEADS TO DEATH WITHIN THE NEXT HOUR	
		ml. per 100 grams of body weight	ml. per 100 sq. cm. of body surface
<i>grams</i>			
96-133	14	$3.82 \pm 0.06^*$	2.03 ± 0.03
180-199	6	3.13 ± 0.07	2.01 ± 0.04
200-219	9	2.88 ± 0.07	1.94 ± 0.05
220-239	42	2.85 ± 0.03	2.01 ± 0.02
240-259	103	2.81 ± 0.02	2.04 ± 0.02
260-279	21	2.76 ± 0.04	2.05 ± 0.03
		Mean: 2.03 ± 0.01	

* Mean and standard error.

TABLE 3

Reduction in bleeding volume owing to respiratory infection

CONDITION	NUMBER OF RATS	BLEEDING VOLUME (ML. PER 100 SQ. CM. OF BODY SURFACE)	"P" VALUE
Normal.....	195	$2.03 \pm 0.01^*$	
Mild respiratory infection.....	19	1.83 ± 0.05	0.01

* Mean and standard error.

secured by calculating the volume of blood to be removed in ml. per 100 sq. cm. of body surface.

c. Previous water intake. Animals deprived of water during the 24 hour fast had slightly smaller but no more uniform bleeding volumes than those having access to water during fasting.

d. Respiratory infection. A mild respiratory infection which was epidemic in the rat colony at one stage in the study brought about a 10 per cent reduction in the bleeding volume of the affected animals and a less uniform response to therapeutic measures (table 3). On these grounds, animals with bleeding volumes 10

per cent or more below the normal average were discarded in subsequent work.

2. *Factors Affecting the Response of Rats to Transfusion.* a. *Position.* In the first series of transfusion experiments the animals were taken down from the slings and placed on their backs in order to expose the jugular vein. When it was found that the change in position favorably influenced the survival time of the animals, this method was abandoned. Thereafter the animals remained in their slings, and transfusions were made into the tail vein as described.

b. *Time of transfusion.* Table 4 illustrates the relation between the survival of rats receiving transfusions of whole blood or of 0.9 per cent sodium chloride solution and the time after bleeding at which the transfusion was given. Both agents were equally effective if they were given at a time, shortly after bleeding, when the respiration rate had increased to 150 cycles per minute, and their effectiveness was much reduced when transfusion was begun at stage 1 or during stages 2 and 3 of the terminal changes in respiration. The data show that a

TABLE 4
Relation between time of transfusion and survival rate

STAGE AT WHICH TRANSFUSION WAS STARTED	NUMBER OF RATS	PER CENT OF RATS SURVIVING 24 HOURS
(A; whole blood: 1.0 ml. per 100 gram of body weight)		
Respiration 150/min.....	5	100
Stage 1.....	10	40
Stages 2 and 3.....	36	3
(B; 0.9 per cent saline: 1.0 ml. per 100 grams of body weight and 2.9 to 3.9 ml. per 100 grams of body weight (for stages 2 and 3))		
Respiration 150/min.....	5	100
Stages 2 and 3.....	8	0

proper comparison of the effects of various therapeutic agents can be made only if they are administered at the same stage in the development of shock in groups of animals. The gasping stage of the terminal respiratory events was chosen as the time for beginning the transfusions because it is a fairly consistent and readily recognizable event, because it takes place in different animals of a series at a fairly uniform time before death may be expected, and because administration at this stage of imminent collapse could be expected to yield the most clear-cut differences in the effects of different therapeutic agents.

c. *Environmental temperature.* There is ample evidence (5-10) that the temperature of the external environment influences the fate of animals in shock. In the course of this work groups of animals were acclimatized for at least a week before use both in the warm room in which the bleeding and transfusion experiments were conducted, at $84 \pm 1^\circ\text{F.}$, and in another room at $73 \pm 3^\circ\text{F.}$ The two groups of animals therefore differed only in the environmental temperatures to which they were exposed before fasting. This difference had no effect on the

bleeding volumes of the two groups of rats, but it did affect their response to transfusions of whole blood and plasma. The data of tables 5 and 6 show that significantly smaller volumes of whole blood or of plasma are required to bring about 50 per cent survival of groups of rats acclimatized at 84°F., and that, for testing therapeutic agents in animals in shock, the temperature at which they are acclimatized as well as the temperature at which the experiments are conducted must be kept uniform if consistent responses to transfusion are to be expected.

3. *The Effects of Transfusion With Blood and Blood Substitutes.* The data of tables 5 and 6 summarize the observations on the effects of transfusions of whole blood and of some blood substitutes on the survival of rats in imminent collapse after acute blood loss. In most instances the response was determined at several dose levels so that the E. D.₅₀ (the dose in ml. per 100 grams of body weight bringing about survival of 50 per cent of treated animals) could be roughly

TABLE 5

The effects of transfusion with whole blood and plasma on the survival of rats, acclimatized at 84°F., in imminent collapse after acute blood loss

NUMBER OF RATS	VOLUME OF TRANS- FUSION (ML. PER 100 GRAMS OF BODY WEIGHT)	PER CENT SURVIVED	AVERAGE TIME OF SURVIVAL	E.D. ₅₀ (ML. PER 100 GRAMS OF BODY WT.)
Whole blood				
18	0.07	0	<i>min.</i> 95	0.86
31	0.80	13	94	
8	1.00	88	58	
2	1.30	100		
Plasma				
8	1.20	25	376	1.20-1.50
5	1.50	100		

estimated. It can be seen that the differences in these values for the several agents used are sufficiently large to indicate clearly their relative effectiveness as transfusion fluids. In one instance in which the number of observations permitted it, the data for the response to whole blood transfusion of rats acclimatized at 84°F. and 73°F. were analyzed by the simple graphical method of Litchfield and Fertig (11), in which the probits of per cent survival are plotted against log. dose. The plots yielded parallel straight lines from which the following estimates of log. E.D.₅₀ and its standard error were made;

$$\text{at } 84^{\circ}\text{F.: Log E.D.}_{50} = -0.068 \pm -0.0101;$$

$$\text{at } 73^{\circ}\text{F.: Log E.D.}_{50} = +0.094 \pm 0.0080.$$

The errors of the estimates of E.D.₅₀ are about 2 per cent, which indicates a satisfactory order of control of the experimental conditions. The method there-

fore may provide a basis for the proper assay of the relative effectiveness of blood and blood substitutes, 1, if a sufficient number of observations are made at dose levels bringing about more than 0 per cent and less than 100 per cent survival,

TABLE 6

The effects of transfusion with whole blood and some blood substitutes on the survival of rats, acclimatized at 73°F., in imminent collapse after acute blood loss

NUMBER OF RATS	VOLUME OF TRANS- FUSION (ML. PER 100 GRAMS OF BODY WEIGHT)	PER CENT SURVIVED	AVERAGE TIME OF SURVIVAL	E.D. ₅₀ (ML. PER 100 GRAMS OF BODY WEIGHT)
50 controls	(not transfused)	0	<i>min.</i> 3	
Whole blood				
5	0.50	0	9	1.24
5	0.75	0	134	
40	1.00	2.5	147	
11	1.20	27	267	
11	1.40	91	1087	
7	1.60	100		
5	2.75	100		
Plasma				
8	1.60	0	112	1.60-2.25
4	2.25	75	360	
3	3.00	100		
Red blood cells in 1.1 per cent sodium bicarbonate				
2	1.30	0	49	2.35-3.00
2	1.70	0	42	
9	2.35	33	111	
4	3.00	75	750	
0.9 per cent sodium chloride				
4	2.90	0	55	3.90-4.60
4	3.90	0	493	
5	4.60	60	172	
4	6.30	100		
2.4 per cent sodium succinate				
5	2.80	0	37	

and 2, if it can be shown that the slopes of the lines relating probit of per cent survival and log dose are not significantly different for different therapeutic agents. Since "survival" must be arbitrarily defined, a significant difference in the slopes of the dose-response curves as well as in E.D.₅₀ for two different agents

would indicate that their action to bring about survival as defined is not the same, and this must qualify any comparisons of E.D.₅₀.

The data of tables 5 and 6 are in good agreement with the observations of other workers on the relative effectiveness of different transfusion media in shock. It is interesting to note that in these circumstances relatively large volumes of a suspension of red blood cells in 1 per cent sodium bicarbonate, and even larger volumes (roughly 3 times the volume of blood lost) of 0.9 per cent saline, may bring about survival of rats in imminent collapse after acute blood loss.

4. *Prolonged Severe Hemorrhagic Shock.* a. *Production.* Rats in imminent collapse after acute blood loss were given a transfusion of heparinized whole blood, about half of the volume required to bring about survival being administered. The animals recovered temporarily, but in one-and-one-half to two-and-one-half hours they again exhibited the respiratory changes characteristic of imminent collapse. At this time, rats acclimatized at 73°F. did not survive even if a volume of blood equal to that originally lost was administered, but rats acclimatized at 84°F. could be revived temporarily by a second transfusion of 1 ml. of whole blood per 100 grams of body weight. These animals collapsed again about 100 minutes later, and at this time a transfusion equal in volume to the blood originally lost did not bring about survival.

b. *Properties.* The animals exhibited symptoms characteristic of prolonged severe hemorrhagic shock. The plasma amino nitrogen increased steadily until death, and there was a terminal fall in the plasma sugar. Animals receiving inadequate transfusion remained in a profound state of prostration until death. They were often hypersensitive to sound or touch. In almost all animals a bloody exudate accumulated round the eyes. In animals autopsied after an inadequate transfusion the skin, muscles, and gastrointestinal tract were found to be cyanotic. From $\frac{1}{2}$ to 7 ml. of a bloody exudate were found in the stomach, and an additional amount, not measured but often copious, was found in the small intestine. In many of these rats, therefore, an amount of fluid equal to at least 2.8 ml. per 100 grams of body weight was lost into the lumen of the gastrointestinal tract. In consequence there was often marked hemoconcentration. In a series of rats the hematocrit rose from a normal value of 45 to values terminally of 47, 48, 54, 55, 55, 56 and 57. Some engorgement of the viscera is indicated by an increase in liver weight from a normal value of 3.00 ± 0.06 gram per 100 of body weight to 3.20 ± 0.04 gram per 100 grams of body weight.

c. *Effects of transfusion.* Table 7 summarizes the observations on the effects of transfusions of whole blood and of saline solution on the survival of rats in prolonged hemorrhagic shock. It will be seen that the rats acclimatized at 73°F. were not revived by a whole blood transfusion equal to 5 per cent of the body weight which brought about survival in all the rats acclimatized at 84°F. The data suggest that transfusion at the 10 per cent level might be effective, but the number of observations is too limited to permit a definite conclusion. It is especially interesting to note that a transfusion of saline solution equal to 10 per cent of the body weight brought about survival in 5 out of 6 rats acclimatized at 84°F.

DISCUSSION. These observations are in agreement with other evidence that

rate of bleeding (12), environmental temperature (5-10), position (13) muscular activity (14) and water intake (15) influence the response of animals to hemorrhage and to transfusion. Three additional factors have been shown to be important in the rat. First, the bleeding volume is a linear function of body surface rather than of body weight, and much more uniform results are secured if the amounts of blood to be removed are calculated in terms of body surface area. This is particularly important if the series of animals used exhibits a wide variation in body weight. Next, the temperature of acclimatization must be kept uniform if consistent responses to bleeding and transfusion are to be obtained.

TABLE 7

The effects of transfusions with whole blood and saline solution on the survival of rats in prolonged hemorrhagic shock

NUMBER OF RATS	VOLUME OF TRANSFUSION (ML. PER 100 GRAMS OF BODY WEIGHT)	PER CENT SURVIVED	AVERAGE TIME OF SURVIVAL
(Rats acclimatized at 84°F.)			
Whole blood			
9	1.5*	0	<i>hours</i> 3.8 ± 3.88†
7	5.0	100	
0.9 per cent sodium chloride solution			
5	5.0	0	1.4 ± 0.83
5	10.0	83	
(Rats acclimatized at 73°F.)			
Whole blood			
19	1.8*	0	1.8 ± 0.72
8	5.0	0	10.8 ± 4.28
3	10.0	66	8.3

* This volume, together with the volume of the transfusion used to prolong the period of shock, equals the volume of blood withdrawn.

† Standard deviation.

Finally the effects of a mild respiratory infection upon the bleeding volume suggest that care should be taken to assure uniform physical condition of the experimental animals. When all of these factors are controlled, it is possible to predict the outcome of bleeding with confidence, a fundamental requirement for testing the effects of blood and blood substitutes.

In the rat, the well-defined terminal respiratory events make it possible to recognize, in a series of animals, the development of comparable critical states after hemorrhage. The uniform responses to transfusion may be ascribed largely to the fact that in the several groups of rats the state of shock at the time

of beginning the transfusions was reasonably uniform. The graphical analysis of the data in one instance suggests that the procedure fulfills most of the requirements of a proper method of assay. Further work must be done, however, with due attention to the number of animals and the dose levels used, before it can be decided that the assay of blood substitutes against whole blood by this procedure is valid. As they stand, the data provide a rough quantitative estimate of the relative effectiveness of the different transfusion fluids used.

The observations on plasma and whole blood are in agreement with current opinion. It is of practical interest to note that a suspension of red cells in 1.1 per cent sodium bicarbonate solution is almost as effective as plasma. Tabor, Kabat, and Rosenthal (16) found that a suspension of red cells in saline solution was as effective as whole blood and more effective than plasma in the treatment of mice after hemorrhage. They ascribe the effects of plasma to its electrolyte content. The data of this study (table 6) indicate that in the rat the effectiveness of plasma is due to other elements than its salts.

Rats in imminent collapse after acute blood loss exhibit most of the metabolic changes that distinguish a fatal from a non-fatal hemorrhage (17). Of these, the most easily determined and reliable one is the change in plasma amino nitrogen, which increases significantly during the bleeding period and rises steadily thereafter until death (18). The other outstanding characteristics of rats in this early stage of severe shock are that a relatively small transfusion of whole blood—equal to about 1 per cent of the body weight—will bring about rapid recovery, and that in animals dying directly or after an inadequate transfusion, the tissues and mucous membranes are bloodless, pale and dry. This agrees with the observations of Werle, Crosby and Wiggers (19) on dogs dying shortly after large rapid hemorrhages.

The production of prolonged hemorrhagic shock by means of small transfusion is comparable with the method of prolonged hypotension used by Werle, Crosby and Wiggers (19) and by Walcott (20) to develop severe hemorrhagic shock in dogs. In both preparations the tissues are engorged, and there is loss of bloody fluid into the gastro-intestinal tract. Both preparations are in "irreversible" shock, in the sense that a transfusion equal in volume to the blood lost does not bring about survival. However, definitions of irreversibility, like those of survival, are necessarily arbitrary. In the rat in prolonged severe hemorrhagic shock transfusions of whole blood of the order of 5 to 10 per cent of the body weight may bring about survival, so that, despite the severity of symptoms, this state of shock while uniform and reproducible, is not in fact irreversible. It is important to emphasize that transfusions which would be regarded as fantastically large in modern clinical practice may bring about recovery from extremely severe states of prolonged shock.

SUMMARY

1. Earlier evidence that rate of bleeding, environmental temperature, position, muscular activity, and water intake influence the response of animals to bleeding

and transfusion has been confirmed. In addition it has been shown that in the rat, *a*, more consistent responses to hemorrhage are obtained if the volume of blood to be removed is calculated in terms of body surface area instead of body weight; *b*, the temperature to which the animals are acclimatized determines the severity of shock developed by means of a standardized bleeding procedure; and *c*, mild respiratory infections may affect the response of animals to bleeding and to transfusion. When all of these factors are controlled the response of rats to hemorrhage can be confidently predicted.

2. In the rat certain characteristic changes in respiration after acute blood loss make it possible to recognize, in a series of animals, the development of comparable states of shock, and to begin transfusions at this stage. The responses are sufficiently uniform to indicate that the method may be used for the assay of the effectiveness of blood and blood substitutes in shock. It is shown that transfusions of relatively large volumes of a suspension of red cells in 1.1 per cent sodium bicarbonate solution or of 0.9 per cent sodium chloride solution may bring about survival of rats in imminent collapse after acute blood loss.

3. Reasonably reproducible states of prolonged severe hemorrhagic shock may be produced by giving one or more small transfusions of whole blood to rats in imminent collapse after acute blood loss. The rats develop an "irreversible" state of shock in the sense that a transfusion equal to the volume of blood lost no longer brings about survival. It is shown that transfusions of a volume of blood of the order of 5 to 10 per cent of the body weight can resuscitate such rats in imminent collapse from prolonged severe hemorrhagic shock.

REFERENCES

- (1) HAIST, R. E. AND J. HAMILTON. *J. Physiol.* **102**: 471, 1944.
- (2) LEE, M. O. AND E. CLARK. *This Journal* **89**: 24, 1929.
- (3) BECKWITH, J. R. AND A. CHANUTIN. *Proc. Soc. Exper. Biol. and Med.* **46**: 66, 1941.
- (4) ROWNTREE, L. G., G. E. BROWN AND G. M. ROTH. *The volume of blood and plasma in health and disease.* W. B. Saunders & Co., Philadelphia and London, 1929.
- (5) BLALOCK, A. AND M. F. MASON. *Arch. Surgery* **42**: 1054, 1941.
- (6) ELMAN, R., M. C. WARREN, JR., C. LISHER AND A. J. MUELLER. *Proc. Soc. Exper. Biol. and Med.* **51**: 350, 1942.
- (7) CLEGHORN, R. A. *Canad. Med. Assoc. J.* **49**: 363, 1943.
- (8) ANTOS, R. J. *Proc. Soc. Exper. Biol. and Med.* **56**: 60, 1944.
- (9) RICCA, R. A., K. FINK, L. I. KATZIN AND S. L. WARREN. *J. Clin. Investigation* **24**: 127, 1945.
- (10) KATZIN, L. I., R. A. RICCA AND S. L. WARREN. *J. Clin. Investigation* **24**: 149, 1945.
- (11) LITCHFIELD, J. T., JR. AND J. W. FERTIG. *Bull. Johns Hopkins Hospital* **69**: 276, 1941.
- (12) ELMAN, R., C. LISHER AND H. W. DAVEY. *This Journal* **140**: 737, 1944.
- (13) RICHARDS, D. W., JR. *Bull. New York Acad. Med.* **20**: 363, 1944.
- (14) ELMAN, D. R. AND H. W. DAVEY. *Proc. Soc. Exper. Biol. and Med.* **56**: 14, 1944.
- (15) LEVINSON, S. O., R. E. WESTONG, M. JANOTA AND H. NECHELES. *Surgery* **12**: 878, 1942.
- (16) TABOR, H., H. KABAT AND S. M. ROSENTHAL. *Pub. Health Reports* **59**: 637, 1944.
- (17) ENGEL, F. L. AND M. G. ENGEL. *This Journal*, **147**: 165, 1946.
- (18) SAYERS, M. A., G. SAYERS, M. G. ENGEL, F. L. ENGEL AND C. N. H. LONG. *Proc. Soc. Exper. Biol. and Med.* **60**: 20, 1945.
- (19) WERLE, J. M., R. S. CROSBY AND C. J. WIGGERS. *This Journal* **136**: 401, 1942.
- (20) WALCOTT, W. W. *This Journal* **143**: 247, 1945.

UREA SYNTHESIS FROM AMINO ACIDS DURING HEMORRHAGIC SHOCK IN THE NEPHRECTOMIZED RAT¹

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In previous reports it has been shown that hemorrhagic shock in the rat is characterized by rising blood levels of amino nitrogen (1) which are due partly to an increased breakdown of protein in the peripheral tissues (2) and partly to a decreased ability of the liver to dispose of amino acids, presumably because of the accompanying anoxia and decreased metabolic activity of this tissue (3, 4). It was implied (1), but not proven, that a depression in the rate of deamination of amino acids by the liver was an important feature of the metabolic picture accompanying shock. Many of these findings have been confirmed in various types of shock by other investigators, both in the rat and other species (5, 6, 7, 8, 9). Van Slyke et al. (9) have in addition reported a decreased urea formation in dogs during hemorrhagic shock.

The present experiments were designed to clarify the nature of the defect in amino acid metabolism by the liver during shock. Since urea synthesis follows closely on deamination in the liver, the rate of this metabolic process has been studied during shock in the rat. A decreased rate of urea synthesis in the face of a mounting amino acid concentration in the blood and liver (1, 10) may be considered as presumptive evidence of a decreased ability to deaminate amino acids, since ammonia (free and loosely bound) has been shown not to increase in the liver during shock (11). It is recognized, of course, that deamination and urea synthesis represent only one metabolic pathway that might be affected. There is now ample evidence that during shock, protein metabolism proceeds in the direction of breakdown of protein to amino acids in the peripheral tissues as well as the liver (2, 10). From this it may be presumed that the reverse reaction of peptide synthesis is probably impaired. Recent studies in this laboratory (11) have shown a depression in the *in vitro* rates of urea synthesis from dl-alanine and from ammonium lactate, with and without ornithine, by liver slices taken from rats in hemorrhagic shock. Urea synthesis under these circumstances was found to be diminished to a greater degree than deamination. However, free ammonia was not found to have accumulated to any marked degree in the livers of shocked rats, as would be expected if urea synthesis were more affected than deamination *in vivo*, suggesting that the relation between the rates of these processes *in vitro* may not necessarily be the same as that *in vivo*.

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METHODS. Male rats of the Sprague-Dawley strain with fasting weights of 210 to 236 grams (average, 220 grams) were used. The rats were nephrectomized 19 to 20 hours before the shock experiments were begun. Water, but no food, was allowed during this period. Experimental procedures were carried out under nembutal anesthesia.

Urea nitrogen was determined on 0.2 ml. of whole blood by a modification of the xanthidrol method of Lee and Widdowson (12) adapted for the Evelyn colorimeter (12a); amino nitrogen by the method of Frame et al. (13). Total urea nitrogen synthesis was calculated on the basis of urea being evenly distributed throughout the body water, i.e., 75 per cent of the body weight.

Amino acids were administered as a casein hydrolysate (Mead Johnson's Amigen) in a 10 per cent aqueous solution containing 11 mgm. total nitrogen and 8 mgm. free amino nitrogen per milliliter. The casein hydrolysate was given intravenously by two methods: *a*, a single injection of 1.5 ml.; *b*, infusion at a constant rate of 1.5 ml. per hour by means of a continuous infusion pump. Since the body weights in each of the experimental groups were kept as uniform as possible, it was not necessary to vary the dosage with body weight.

The rats were bled from the cut tail in volumes equivalent to 3 per cent of the body weight plus the volume of injected amino acid solution.

RESULTS. The interpretation of changes in the blood urea of shocked animals, as compared to normal ones, is complicated by the effects of alterations in renal function during shock. This difficulty was avoided by using nephrectomized rats. The rate of rise of blood urea nitrogen in the nephrectomized animal is a measure of the rate of protein catabolism of the tissues and of urea synthesis by the liver.

To estimate the rate of urea formation prior to bleeding, the rats were nephrectomized 19 to 20 hours before other experimental procedures were carried out. In a series of 46 rats, the average hourly increase in total urea nitrogen during the first 20 hours after nephrectomy was 6.6 ± 0.18 mgm., or 3.0 mgm. of nitrogen per 100 grams of body weight, per hour. This value corresponds closely with the hourly rate of nitrogen excretion in the urine previously observed in this strain of rats in this laboratory and indicates that this is a reliable method of measuring protein metabolism. Nephrectomy did not influence the level of blood amino nitrogen.

Preliminary determinations of blood urea levels after hemorrhage in nephrectomized rats showed no consistent alterations, compared to those in control nephrectomized rats. However, since the rise in urea N is small in the controls (3-4 mgm. per cent per hour), a decrease in rate of urea formation might be obscured in the shocked rat because of the great increase in amino acid substrate for the liver to handle during shock. For not only is there an increase in the amino acid content of the blood flowing through the liver, but also there is an increase of as much as 25 per cent in the amino nitrogen content of the liver tissue itself during shock (10). It is clear, therefore, that a substantially depressed maximal capacity for urea formation could still be present during shock without evidence of decreased urea production.

For these reasons, the rates of urea formation from administered amino acids were compared in control and shocked nephrectomized rats. A measure was given thereby of the amount of urea which the normal liver could synthesize when an increased amount of amino acids was delivered to it. It was decided to administer a mixture of amino acids, since this seemed to be a more physiological process than the flooding of the organism with a single amino acid. Preliminary observations were made on the effects of casein hydrolysate given by stomach

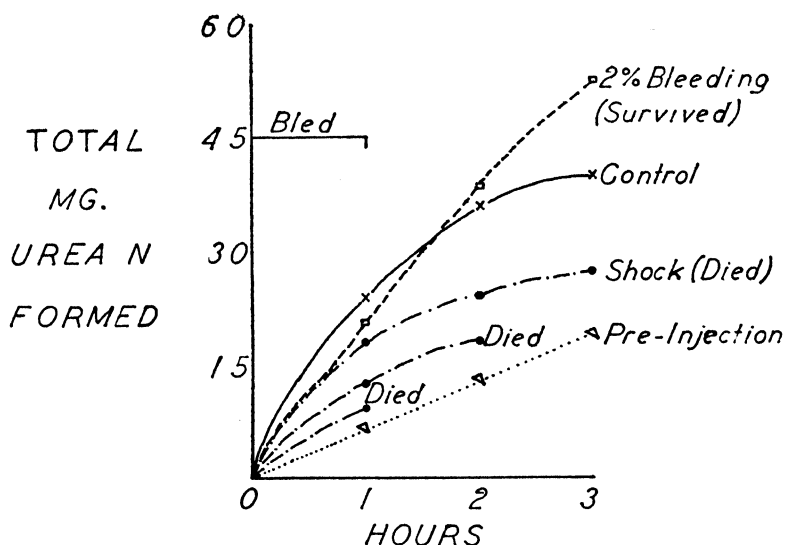


Fig. 1. The effects of a single intravenous injection of 1.5 ml. of 10 per cent casein hydrolysate (16.5 mgm. N) on urea formation in nephrectomized rats. The amino acids were injected rapidly at the outset. The 18 shock rats (·—·—·) were bled 3 per cent of their body weight plus 1.5 ml. during the first hour. Six died in 3 hours, 7 in two hours, and 5 in one hour. The sublethal hemorrhage group (□ — — — □) included 6 rats that were bled 2 per cent of their body weight in one hour, and all survived. Pre-injection (Δ . . . Δ) represents the average normal hourly rate of urea formation prior to injection of amino acids. The difference between the pre-injection curve and all others measures the excess in urea formed from the injected amino acids and from the products of protein catabolism. The control series (× — — — ×) included 10 rats.

tube, but the results were complicated by irregularities in the rate of absorption of amino acids in both the normal and the shocked rats.

Figure 1 illustrates the effects of a single intravenous injection of 1.5 ml. of 10 per cent casein hydrolysate on the subsequent accumulation of urea nitrogen in control and bled nephrectomized rats. The casein hydrolysate was injected rapidly into the saphenous vein, and bleeding was carried out over the course of the next hour. After being injected rapidly in this manner, the animals usually hyperventilated for a few minutes and sometimes showed some spasmodic movements of the limbs. In the figure, the pre-injection rate of synthesis of urea nitrogen is represented by the dotted line and is calculated on the basis of the average amount of urea nitrogen formed per hour in the 20 hours following

nephrectomy and prior to injection. The curves for the control and bled rats represent the total accumulated rise in urea nitrogen for three hours, including both that coming from the injected amino acids and that which would be expected from the tissue amino acids in that period. The extra urea formation can be readily estimated by subtracting the pre-injection rate in each case. For the 10 control animals the average total increase in urea nitrogen is represented by the solid curve in figure 1. There was a marked increment in urea synthesis during the first hour, only a small addition in the second hour, and in the third hour there was no excess over the expected normal hourly rise, as can be estimated by comparing the slope with that of the pre-injection curve. Eighteen rats were subjected to fatal hemorrhage, 6 succumbing in 3 hours, 7 in 2 hours, and 5 in 1 hour. The results are represented in figure 1 by the dot-and-dash curves. In all three groups the amount of urea formed hour by hour was less than the controls, and the more severe the shock, the less was the urea formed. However, in the rats surviving three hours, the decrease in total urea formation was not statistically significant ($p = 0.07$). This is not unexpected, since the major increment in urea formation in the controls occurred in the first hour, while in the bled series the degree of shock as measured by the rise in blood amino nitrogen was not appreciable at the end of the first hour. In the rats that died in 1 and 2 hours the decreases in urea formation were statistically significant.

It is well known that in various stress situations, or even in animals surviving shock severe enough to have caused renal shut-down, there is an increased urinary excretion of urea nitrogen. This is generally attributed to the increased protein catabolism associated with stress and shock. Since this observation is paradoxical in light of the demonstration of *decreased* urea synthesis during severe shock, a series of 10 rats were subjected to 2 per cent hemorrhage to demonstrate the effect of sublethal bleeding on urea formation. Four of these rats showed a rise in blood amino nitrogen at the end of bleeding, indicating hepatic damage, so were discarded. The remaining six, charted in figure 1 by the broken line, had no significant elevations in amino nitrogen during the experiment, so may be considered as examples of hemorrhage without evidence of hepatic failure. All survived. It will be noted in figure 1 that the urea synthesis in this group was not appreciably different from that of the control group in the first hour, but in contrast to the latter, continued at an almost undiminished rate during the following two hours, the increments in these periods being significantly greater than the controls ($p = 0.03$). Not only was the total amount of urea formed in the 2 per cent bleeding group greater than that in the control group, but it was also greater than could be accounted for by complete conversion of injected nitrogen to urea N. Sixteen and five-tenths milligrams of N were injected. Fifty-one and seven-tenths milligrams of urea N were formed in 3 hours, of which 19.8 mgm. may be accounted for from the pre-injection rate of 6.6 mgm. per hour, leaving 31.9 mgm. as newly formed urea N. Even if the injected 16.5 mgm. N were all converted to urea N, which is unlikely, an excess of 15.4 mgm. urea N is left, representing urea synthesis by the liver from amino acids coming from the tissues. This demonstrates that when hemorrhage is not severe enough to result in shock

and to embarrass hepatic function, an excess of protein catabolism products coming from the periphery is readily converted into urea. Urea synthesis following hemorrhage thus has a biphasic character, with an increase possible in the early stages before shock has developed and a decrease in the later stages when liver function fails and the animal is in shock.

It should be noted in passing that in these experiments the urea synthesis from the injected amino acids in the controls was also slightly greater than would be expected from 100 per cent conversion of the injected nitrogen to urea nitrogen. This is undoubtedly due to the effects of the rapid injection of amino acids, which is known to cause a sympathetic stimulation (14) and which would thus serve as a degree of stress.

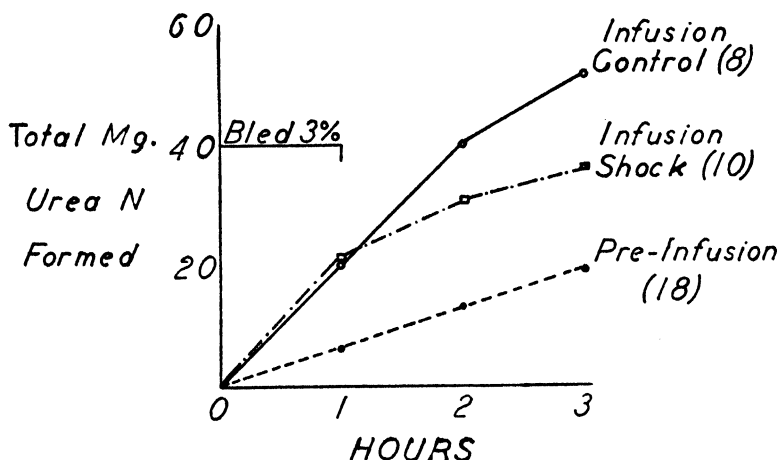


Fig. 2. The effect on urea formation of continuous intravenous infusion of a 10 per cent casein hydrolysate solution at the rate of 1.5 ml. (16.5 mgm. N) per hour in control and fatally bled nephrectomized rats. The pre-infusion curve represents the average rate of urea formation prior to the infusion. The numbers in parentheses refer to the number of rats in each experimental group.

The stimulating effect of the rapid injection of amino acids may be reduced, and a sustained rate of extra urea synthesis over several hours may be obtained by continuous intravenous infusion of casein hydrolysate at the rate of 1.5 ml. (16.5 mgm. N) per hour for three hours. Figure 2 records the results of a series of such infusions. The pre-infusion rate of urea formation was calculated as in the previous figure, and the difference between it and the experimental curves represents the extra urea formation from the infused amino acids. In the control series the urea increased rapidly during the first two hours but slackened off in the third, despite the fact that the same amount of amino acid was infused during each hour. This suggests that the initial tendency is to convert the amino acids to urea, but that after two hours a different metabolic pathway becomes more prominent, probably in the direction of the synthesis of protein. An initial stimulating effect of the infusion itself on protein catabolism, as in the previous

experiment, cannot be ruled out. The shock series consisted of 10 rats which survived approximately 3 hours and were bled 3 per cent plus 1.5 ml. during the first hour and 1.5 ml. each hour thereafter. The urea formation during the first hour was identical with that of the control group but fell off to a significant degree during the last two hours. Figure 3 illustrates the course of the blood amino nitrogen during these experiments. During the first hour there was no difference in the blood amino nitrogen of the two groups, but thereafter the bled rats showed the characteristic rapid accumulation of amino nitrogen until death. Figure 4 shows the relationship during shock between the rise in blood amino nitrogen and the milligrams of urea nitrogen formed per hour. A highly significant negative correlation was found with a correlation coefficient of -0.715 . The higher the blood amino nitrogen rose, the smaller was the amount of urea synthesized.

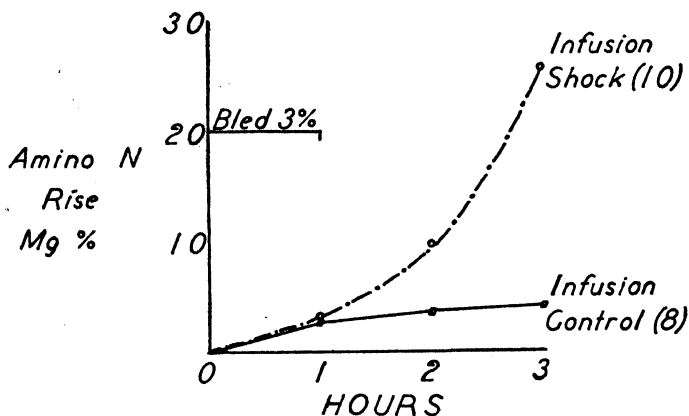


Fig. 3. The effect on the blood amino nitrogen of continuous intravenous infusion of a 10 per cent casein hydrolysate solution in control and bled nephrectomized rats. Amino nitrogen plotted as milligram per cent rise over pre-infusion levels.

Figure 5 illustrates the proportion of infused nitrogen converted to urea each hour by the control and shock rats. The difference between the control and the shock series was most striking in the second hour. In the third hour the percentage of nitrogen converted to urea decreased sharply in the control. However, in the shocked rats no urea was formed from the injected nitrogen during this hour. Considering that the blood levels of amino nitrogen in the shocked rats were 21 mgm. per cent higher than in the control series at this time (fig. 3), this represents a substantial decrease in the liver's ability to synthesize urea from amino acids. The recorded percentages of nitrogen converted to urea are calculated on the assumption that the amounts of urea arising from conversion of body nitrogen remain relatively constant during the three hours of observation. As was indicated in the injection experiments, this may not be strictly true in the control series. In the shock series there is undoubtedly a contribution of nitrogen from the tissues so that the percentage of urea formed from available nitrogen is certainly even lower than is indicated by the chart.

DISCUSSION. The combination of a rising blood level of amino nitrogen with a declining rate of urea synthesis in the absence of any appreciable accumulation of ammonia nitrogen in the liver (11) strongly supports the previously presented view (1) that one of the defects in liver metabolism during shock is a decreased ability to deaminate amino acids. The normal liver responds by a sharp increase in urea production when amino acids are supplied to it either by injection or by an increased release of the products of protein catabolism, as in sublethal hemorrhage. Indeed, in view of the already demonstrated large release of amino nitrogen by the peripheral tissues during shock (2), the rate of urea formation by the sublethally bled rats given amino acids by vein (fig. 1) might legitimately be

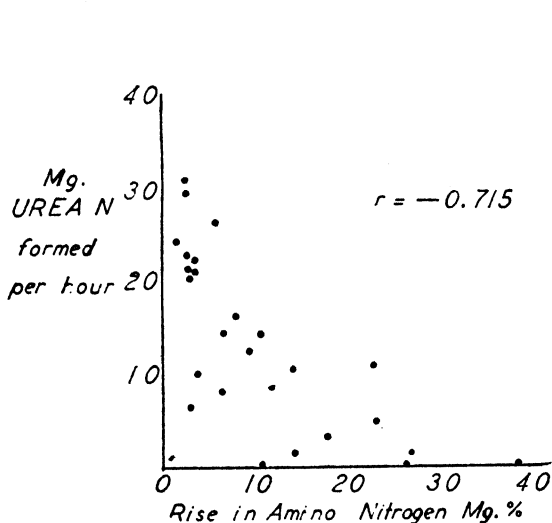


FIG. 4

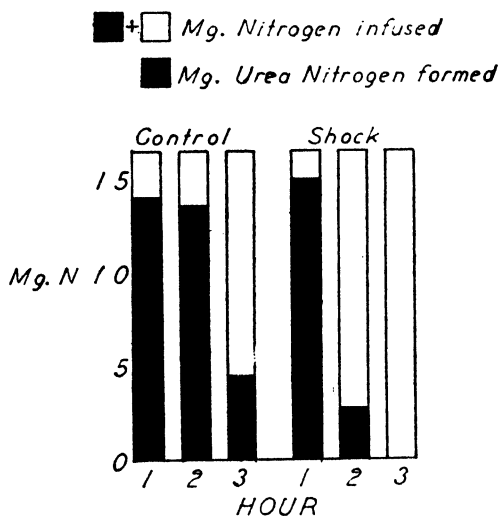


FIG. 5

Fig. 4. The relation between the milligrams of urea nitrogen formed and the rise in blood amino nitrogen in hemorrhagic shock during the continuous intravenous infusion of a 10 per cent casein hydrolysate solution. $r = -0.715$ is the correlation coefficient and indicates a highly significant negative correlation.

Fig. 5. The proportion of infused nitrogen from the casein hydrolysate converted to urea nitrogen in each hour in the control and bled nephrectomized rats.

used as a control rather than the rate in the normal nephrectomized animals given amino acids. This would emphasize the urea-forming capacity of the normal liver and the degree of disability in the shocked animals.

The results described here are in good agreement with the *in vitro* studies of Wilhelmi et al. (11), with regard to depression of urea formation in hemorrhagic shock. Although the *in vitro* rate of deamination did not appear to be as greatly influenced as the synthesis of urea in their experiments, our results, taken in conjunction with their data on the blood and liver ammonia nitrogen, support their conclusion that the *in vivo* rates of deamination may be less than the *in vitro* work implied.

One might inquire at this point whether the observed decrease in urea synthesis

by the liver is due simply to the decreased blood flow through the liver and hence to a decreased clearance of amino acids, or whether it represents a true defect in the liver's ability to metabolize amino acids in shock. While a decreased clearance undoubtedly is a factor, the evidence points to a true metabolic defect being more important. This conclusion is supported by the observation of decreased oxygen consumption and urea synthesis by liver slices *in vitro*, in circumstances in which blood flow is no longer a factor, and by the fact that there was no accumulation of urea nitrogen in the liver (11) during shock. If urea formation were proceeding at a normal rate in the liver during shock, one would anticipate a higher than normal urea content when the amino nitrogen content is increased and blood flow decreased.

It should be emphasized that these results do not in any way contradict the well-known observation of an increased urea nitrogen excretion in certain cases of hemorrhage, trauma, burns, etc., and almost all cases of shock that have been treated and have recovered. An increased rate of protein catabolism by the anoxic tissues is probably a constant feature of all types of shock and begins soon after the shock-inducing processes have been started. Whether these protein catabolism products can be converted to urea and at what rate depends entirely on the degree to which the liver has become anoxic. Evidence has already been presented (3, 15) that even when kept totally anoxic for as long as an hour, the rat liver still retains considerable recuperative power as far as its ability to metabolize amino acids is concerned. The marked suppression of urea formation described in this report represents a severe degree of damage to liver function and correlates well with the evidence already presented of a poor prognosis when blood amino nitrogen accumulates beyond a certain degree (16).

From the data on the rat which have been accumulated in this laboratory during the past few years, it now seems possible to make a distinction in a biochemical sense between the effects of hemorrhage from which the animals may recover spontaneously and of hemorrhage severe enough to result in shock with a fatal outcome if no therapy is given. The first group is characterized by little or no rise in the blood amino nitrogen despite an increased rate of protein catabolism by the peripheral tissues. The liver remains competent, as judged by its ability to synthesize urea, both from injected amino acids and from the products of increased protein catabolism, by its maintenance *in vitro* of relatively normal rates of oxygen uptake, deamination, and urea synthesis, and by its normal composition with respect to water and electrolytes (17) and amino nitrogen (10, 11). The liver glycogen begins to fall in this stage, while the blood sugar, lactate, and pyruvate rise, but a normal lactate/pyruvate ratio is maintained (1, 2). The adrenal cholesterol and ascorbic acid concentrations decrease almost immediately after bleeding has begun (18), but both return towards normal, the cholesterol after about 7 hours and the ascorbic acid within two hours. This is the characteristic response of the adrenal cortex to mild or moderate stress. The liver ascorbic acid content increases, while that of the blood shows little change (18).

The shock phase is distinguished by a progressive elevation of the plasma and liver amino nitrogen (11, 16). The liver shows a decreased ability to deaminate

amino acids and to synthesize urea, both *in vivo* and *in vitro*, a decreased rate of oxygen consumption (4), a marked alteration in its water and electrolyte composition (17), and an increased amino nitrogen content without appreciable change in the ammonia and amide nitrogen (10, 11), all indicating impairment of its function. This occurs at a time when the blood pressure has fallen and remains below about 80 mm. of mercury and the portal venous oxygen saturation has decreased by 50 per cent or more (1, 3). The blood sugar starts to fall because of depletion of liver glycogen and because of increased rates of glycolysis and of glucose utilization in muscle. The lactate/pyruvate ratio in the blood increases owing to the more rapid accumulation of lactate, indicating a shift towards the anaerobic metabolism of carbohydrate (1, 2). The adrenal cholesterol and ascorbic acid contents reach very low levels and show no tendency to recover (18). The liver ascorbic acid content declines sharply, while the blood level increases (18). The level of the plasma amino nitrogen appears to be the most reliable single indication in the rat that shock has developed.

The initial biochemical changes after hemorrhage are largely responses to stress in general and probably represent compensatory reactions in the same sense as the initial circulatory and respiratory changes that follow bleeding. The later changes mirror the effects of continued anoxia on organ and tissue metabolism and function. Although there is clear-cut evidence of marked disturbance of hepatic function during shock, it is not possible to state certainly that this failure is itself a critical factor in the outcome. However, the recent observation by Fine (19) that dogs brought to the stage of irreversible shock after hemorrhage may be saved by viviperfusion of blood through the liver but die if the same volume of blood is administered through a systemic vein, strongly suggests that the maintenance of liver circulation and function is of great importance.

SUMMARY

The effect of hemorrhage on the rates of urea formation from an intravenously injected amino acid mixture has been studied in nephrectomized rats.

Fatal hemorrhage with elevated blood levels of amino nitrogen consistently was associated with a decreased rate of urea formation from injected amino acids. There was a highly significant *negative* correlation between the levels of blood amino nitrogen and the amounts of urea formed from infused amino acids during shock.

Non-fatal hemorrhage with no change in blood amino nitrogen was associated with an increased production of urea. This is interpreted as due to the conversion to urea by a still normal liver of the products of protein catabolism from the peripheral tissues, in addition to the infused amino acids.

The view is presented that the decreased rate of urea formation in shock is not simply the result of a decreased clearance of amino acids through the liver, but represents a true metabolic defect in this organ.

Biochemical distinctions between hemorrhage from which spontaneous recovery is possible and hemorrhagic shock are considered.

REFERENCES

- (1) ENGEL, F. L., M. G. WINTON AND C. N. H. LONG. *J. Exper. Med.* **77**: 397, 1943.
- (2) RUSSELL, J. A., C. N. H. LONG AND F. L. ENGEL. *J. Exper. Med.* **79**: 1, 1944.
- (3) ENGEL, F. L., H. C. HARRISON AND C. N. H. LONG. *J. Exper. Med.* **79**: 9, 1944.
- (4) RUSSELL, J. A., C. N. H. LONG AND A. E. WILHELMI. *J. Exper. Med.* **79**: 23, 1944.
- (5) CRAIG, F. N. *J. Biol. Chem.* **150**: 209, 1943.
- (6) GLENN, W. W. L., J. MUUS AND C. K. DRINKER. *J. Clin. Investigation* **22**: 451, 1943.
- (7) HOAR, W. S. AND R. E. HAIST. *J. Biol. Chem.* **154**: 331, 1944.
- (8) KLINE, D. L. *Fed. Proc.* **4**: 41, 1945
- (9) VAN SLYKE, D. D., R. A. PHILLIPS, P. B. HAMILTON, R. M. ARCHIBALD, V. P. DOLE AND K. EMERSON, JR. *Trans. Ass. Am. Physicians* **58**: 119, 1944.
- (10) RUSSELL, J. A. AND C. N. H. LONG. *This Journal* **147**: 175, 1946.
- (11) WILHELMI, A. E., J. A. RUSSELL, M. G. ENGEL AND C. N. H. LONG. *This Journal* **144**: 74, 1945.
- (12) LEE, H. L. AND E. M. WIDDOWSON. *J. Biochem.* **31**: 2035, 1937.
- (12a) ENGEL, M. G. AND ENGEL, F. L. To be published.
- (13) FRAME, E. G., J. A. RUSSELL AND A. E. WILHELMI. *J. Biol. Chem.* **149**: 255, 1943.
- (14) BASILIOU, B. AND F. ZELL. *Biochem. Ztschr.* **238**: 418, 1931.
- (15) WILHELMI, A. E., J. A. RUSSELL, F. L. ENGEL AND C. N. H. LONG. *This Journal* **144**: 669, 1945.
- (16) SAYERS, M. A., G. SAYERS, M. G. ENGEL, F. L. ENGEL AND C. N. H. LONG. *Proc. Soc. Exper. Biol. and Med.* **60**: 20, 1945.
- (17) DARROW, D. C. AND F. L. ENGEL. *This Journal* **145**: 32, 1945.
- (18) SAYERS, G., M. A. SAYERS, T. Y. LIANG AND C. N. H. LONG. *Endocrinology* **37**: 96, 1945.
- (19) FINE, J., H. A. FRANK AND A. M. SELIGMAN. *Ann. Surg.* **122**: 652, 1945.

AMINO NITROGEN IN LIVER AND MUSCLE OF RATS IN SHOCK AFTER HEMORRHAGE¹

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The characteristic increase in plasma amino nitrogen which occurs in rats in shock after hemorrhage has been considered to be due partly to failure of liver function and partly to the release of amino acids from peripheral tissues (1, 2). Some increase in the average concentration of free amino nitrogen was found in the livers of an unselected group of rats after hemorrhage (3). Since the amino nitrogen content of the livers of some of the animals was too high to be the likely result only of an increase in the plasma amino nitrogen, the hepatic origin of some of the amino compounds was tentatively suggested. To confirm these observations, simultaneous determinations of the plasma and liver amino nitrogen levels have now been made. In addition, the free amino nitrogen of a typical muscle (gastrocnemius) has also been estimated.

If a tissue in which an increase of amino nitrogen content occurs is itself the source of the material released, an increase in the amount and concentration of intracellular amino nitrogen may be expected. Since a normal distribution of extra and intracellular water cannot be assumed in the tissues of animals after severe hemorrhage, the extracellular fluid volumes of the liver and muscle have been estimated by comparison of the plasma and tissue sodium levels in each instance, and calculated values for the amino nitrogen concentration in intracellular water have been obtained. The apparent intracellular amino nitrogen content of the tissues could be affected by any considerable retention of blood, since the red cells contain much less amino nitrogen than do liver or muscle cells; significant changes in blood content might be anticipated in these circumstances in liver, but probably not in muscle. Estimations of the hemoglobin content of the livers and of the hemoglobin and amino nitrogen concentrations in red cells were therefore made also, and corrections applied to the figures for the intracellular amino nitrogen and water of the livers. As it happened, these corrections were quite small in all the experiments reported here.

In severe shock the water content of the liver increases, and some variation in the hydration of other tissues may also be expected. Therefore, for accurate comparison of the composition of the tissues, it was necessary to refer the values obtained to the dry weight of the tissues. In the case of the liver, the fat-free dry weight was used as the basis of calculation.

METHODS. Fasted male rats of the Sprague-Dawley strain, weighing from 180 to 250 grams, were used in these experiments. Shock was induced by the

¹ The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research & Development and Yale University.

removal of 2.6 to 3.0 (average, 2.9) per cent of the body weight of blood from the cut end of the tail in the course of about one hour. The animals were bled under light pentobarbital anesthesia; usually no additional anesthetic was required after the initial injections. The samples for analysis, blood from the vena cava, the gastrocnemius muscles, and the whole livers, were taken from the anesthetized animals usually from one to two hours after the end of bleeding. At this time most of the bled animals were in deep shock, as judged by the character of the respiration, pallor, and feebleness of reflex responses, but only two of the animals were obviously moribund at the time of sacrifice. Two other bled rats were still in good condition at three hours after the end of bleeding, requiring additional barbiturate before sacrifice, and were judged to be only in the early stage of shock. The data obtained from these animals are given separately in tables 1 and 2.

TABLE 1
Sodium and water in tissues of rats in shock after hemorrhage

	NO. OF EXP.	PLASMA SODIUM mM	TISSUES		
			Sodium	Total water	Extracellular water
			Liver—per 100 grams fat-free dry weight		
			m.mol.	grams	grams
Control rats.....	9	146 ± 4	10.7 ± 0.1	278 ± 3	68 ± 2
Bled rats					
Moderate shock.....	2	143	14.5	288	94
Deep shock.....	9	159 ± 2	25.4 ± 1.6	316 ± 6	149 ± 11
			Muscle—per 100 grams dry weight		
Control rats.....	9		7.3 ± 0.1	318 ± 2	47 ± 1
Bled rats					
Moderate shock.....	2		7.5	317	48
Deep shock.....	9		7.4 ± 0.2	316 ± 3	43 ± 2

Control rats were maintained under light pentobarbital anesthesia for varying lengths of time before sacrifice.

The following determinations were made on each rat: whole blood amino nitrogen, hematocrit, and hemoglobin: plasma amino nitrogen and sodium; liver amino nitrogen, sodium, hemoglobin, water, and fat (ether extractable); and muscle amino nitrogen, sodium, and water. From these values were calculated in each instance the extracellular content of amino nitrogen and of water in the liver and muscle, the amino nitrogen and hemoglobin content of the red cells, and thence the amino nitrogen and intracellular water of the red cells in the liver, and finally, the concentration of amino nitrogen in the liver cell water and in the intracellular water of the muscle samples. In making these calculations it was assumed that plasma contained 92 per cent water, the red cells 65 per cent water, and that all of the sodium was extracellular in the blood, liver and muscles. The concentrations of the various substances were expressed in terms of the fat-free dry material in the liver and of dry material in muscle.

Amino nitrogen determinations were made by the colorimetric method (4) on tungstic acid filtrates made from 0.2 ml. samples of blood and plasma and from 0.2 gram samples of fresh tissue. In the preparation of the latter, the tissue samples were first fixed in 0.2 ml. portions of $\frac{2}{3}$ N sulfuric acid, then worked to a paste with a glass rod (liver) or finely minced with scissors (muscle). Water (about 9 ml.) and 0.2 ml. of 10 per cent sodium tungstate were added in order, each with thorough mixing. The samples were then made to 10 ml. volumes and allowed to stand with occasional stirring for about one hour before centrifugation. One or 2 ml. of the filtrate were used for the estimation of amino nitrogen.

Hemoglobin was determined colorimetrically as the alkaline oxyhemoglobin (Evelyn). Liver samples (0.5 gram) were ground to a pulp, made to 10 ml. volumes with 0.01 M phosphate buffer, pH 7.2, and extracted one hour in the cold. After centrifugation 3 ml. samples of the turbid supernatant were parti-

TABLE 2

Amino nitrogen in tissues of rats in shock after hemorrhage

	NO. OF EXP.	PLASMA AMINO NITROGEN	TISSUE AMINO NITROGEN			AMINO NITRO- GEN IN INTRACELLULAR WATER
			Total	Extracellular	Intracellular	
		<i>mgm. per 100 ml.</i>	<i>Liver—per 100 grams fat-free dry weight</i>			<i>Liver</i>
			<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm. per 100 ml.</i>
Control rats.....	9	7.4 ± 0.5	240 ± 10	5 ± 0.4	231 ± 10	115 ± 5
Bled rats						
Moderate shock.....	2	10.1	272	10	259	140
Deep shock.....	9	17.4 ± 1.5	301 ± 10	26 ± 3.5	271 ± 10	172 ± 9
			<i>Muscle—per 100 grams dry weight</i>			<i>Muscle</i>
Control rats.....	9		250 ± 11	4 ± 0.3	245 ± 11	91 ± 4
Bled rats						
Moderate shock.....	2		274	5	272	102
Deep shock.....	9		289 ± 6	7 ± 0.5	281 ± 5	103 ± 2

ally clarified by the addition of 0.2 ml. of concentrated ammonium hydroxide and made to 10 ml. with water. The light absorption was measured with filter 540 as usual; then the color of the hemoglobin was bleached by the addition of 0.2 ml. of concentrated hydrogen peroxide, and the light absorption of the mixture again determined 10 to 20 minutes later. The difference in absorption was taken as a measure of the hemoglobin present in the liver tissue.

Sodium determinations were made as described by Leva (5), on trichloroacetic acid filtrates of plasma (0.1 ml.) or of tissue (0.3 to 0.5 gram). In this method manganous uranyl sodium acetate is formed, followed by the colorimetric determination of the manganese as permanganate. Excellent recoveries of sodium were obtained, provided that precipitation and washing of the triple salt were carried out at temperatures below 24°C. At any higher temperatures, it was necessary to use solutions containing more uranyl acetate than recommended by

Leva (85 grams per liter instead of 80 grams). It was not found necessary to remove potassium or phosphate ions before the precipitation of the sodium salt.

RESULTS AND DISCUSSION. The principal data obtained in these experiments are presented in tables 1 and 2. At the time the samples were taken, the bled rats all showed a considerable elevation of the total amino nitrogen levels of the liver, the average of the group in severe shock being 25 per cent above the control value. The increase in muscle amino nitrogen, 15 per cent above the normal average, was also significant ($t = 3.22$). As was to be expected from previous work, the plasma amino nitrogen was high in the bled animals, ranging from 12 to 25 mgm. per cent in the animals in severe shock. In the two rats in mild shock, the plasma amino nitrogen was only slightly above the normal range. The elevations in tissue amino nitrogen were in these experiments much too great to have been the result only of increasing plasma amino nitrogen concentrations. If the proportion of plasma in the tissues had remained normal, the average increase in the amino nitrogen of the liver due to plasma would have been only about 8 mgm. per 100 grams dry weight or 3 per cent of the normal level. As indicated below, a considerable increase in the plasma volume appears to have occurred in the liver, but the maximum possible figure for the extracellular amino nitrogen, given in table 2, could account for only one-third of the increase. In muscle only a small fraction of the amino nitrogen was extracellular in both the normal and bled rats. The increase in liver and in muscle amino nitrogen in the bled animals may therefore be considered real.

In the livers of the bled animals, the sodium concentrations were very much increased. This observation confirms that of Darrow and Engel (6), who present in one of their series data obtained in conditions quite similar to those used here. The total water of the liver was also increased in the present experiments, but to a much smaller extent than was the sodium. The calculated extracellular water volume was therefore quite high, and the intracellular water diminished. The average concentration of amino nitrogen in the intracellular water was 50 per cent above normal in the animals in severe shock. It is not certain, of course, that all of the sodium remained extracellular in these circumstances. Some of the sodium may have entered the liver cells in exchange with potassium, as a result of changes in the functional integrity of the cells and consequent alterations in their permeability. However, as Darrow and Engel have remarked, the accompanying large increase in liver chloride concentration which they observed makes it seem more likely that most of the sodium in fact remained in the extracellular fluid, and that calculations based on the sodium content of the liver present a reasonable view of the water distribution in it. The calculated amounts of extracellular water and amino nitrogen are maximal; the estimate of the concentration of amino nitrogen in the intracellular water of the liver of the bled animals may therefore be too high, but the qualitative change can be regarded with confidence.

The contribution of red blood cells in the liver to the total amino nitrogen of the liver was not large in any of these experiments and was similar in the two series. The average hemoglobin concentration in the liver was 1.1 ± 0.1 per

cent of the wet weight in both cases. The calculated concentration of amino nitrogen in the red cells of the blood averaged 23.5 ± 0.8 mgm. per 100 ml. in the control and 29.8 ± 1.1 mgm. in the bled rats. The contribution of red cell amino nitrogen to the total liver amino nitrogen (from the ratio of hemoglobin in the liver to hemoglobin in the red cells, times the amino nitrogen concentration in the red cells) was then 3 to 4 mgm. per 100 grams dry weight. The red cell water in the liver was taken to be 65 per cent of the red cell volume, averaging 10 grams per 100 grams dry weight of liver. Since the amount of hemoglobin was so small and so uniform, the alterations made by the red cell corrections in the values for concentration of amino nitrogen in liver cell water were not important in these experiments. However, in other circumstances as, for instance, in shock due to other types of stress than hemorrhage, it is conceivable that much more blood might be retained by the liver. In this case dilution of the tissues by red cells, containing much less free amino nitrogen than do liver cells, would tend to reduce any increase in liver amino nitrogen.

It has been assumed in making the calculations discussed here that the amino nitrogen concentration of plasma from the venous blood was the same as that of the plasma and intracellular fluid in the tissues. For the muscles, this assumption was probably nearly true. With respect to the liver, more accurate estimations would have been possible if plasma had been available from portal vein blood. Since the portal circulation is seriously reduced in shock, the intestinal tract may become a source of free plasma amino nitrogen, and the extracellular amino nitrogen of the liver may be therefore higher than the systemic plasma level indicates. However, in order to account for all of the increase in liver amino nitrogen as plasma amino nitrogen, the increase in portal plasma concentration would have had to be several times that which was observed in plasma from the vena cava. In a few unpublished experiments Engel and Harrison, in this laboratory, found the changes in amino nitrogen in whole blood from the portal vein of rats during shock to be not greatly different from those observed in blood from the tail. It does not seem likely then that any large error has been introduced here by the use of systemic plasma amino nitrogen levels.

The amount of intracellular amino nitrogen in the muscles was also increased significantly in the bled animals ($t = 3.16$). The difference in concentration of amino nitrogen in the intracellular water was somewhat less marked ($t = 2.76$; $p = 0.02$). Although there was no change in the amounts of sodium or of water in this tissue, the extracellular water was somewhat low, since the average plasma sodium was increased; but this reduction was of doubtful significance. Therefore, a minimum value was probably given to the difference between the two series in the amino nitrogen concentration of intracellular water of muscle.

The increase in intracellular concentration of amino nitrogen in the liver and muscle in severe hemorrhagic shock must be the result of a disturbance in the normal balance between formation and breakdown of cellular protein. It is probably to be ascribed to the relative failure of oxygen supply to all the tissues and particularly to the liver in this condition. Evidence of an increase in the

rate of contribution of amino acids by peripheral tissues was previously obtained after hemorrhage in eviscerated rats (1). When the circulation is restored to the liver after a period of anoxia *in vivo*, the plasma amino nitrogen rises more rapidly for a time than it does in similar animals in which the liver remains out of the circulation (2), indicating the release of amino acids by the anoxic liver. It may be that in severe shock also there is a net contribution of amino acids to the plasma by the liver rather than removal of them. In any case, the liver not only fails in the catabolism of amino acids (7), despite a plethora of substrate, but the relative rate of assimilation of amino acids must also be reduced to some extent in hemorrhagic shock.

SUMMARY

In rats in shock after hemorrhage, the concentration of free amino nitrogen rises in the liver and in muscle (gastrocnemius). This increase is not due to the inclusion of plasma of high amino nitrogen content but is mainly in the intracellular compartment. These data confirm previous indications of an increase in the rate of release of amino acids from muscle, and show that in the liver also the normal balance between formation and breakdown of cellular protein is disturbed. These two factors, as well as a relative failure in the catabolism of amino acids, then contribute to the increase in plasma amino nitrogen which occurs in hemorrhagic shock.

REFERENCES

- (1) RUSSELL, J. A., C. N. H. LONG AND F. L. ENGEL. J. Exper. Med. **79**: 1, 1944.
- (2) ENGEL, F. L., H. C. HARRISON AND C. N. H. LONG. J. Exper. Med. **79**: 9, 1944.
- (3) WILHELMI, A. E., J. A. RUSSELL, M. G. ENGEL AND C. N. H. LONG. This Journal **144**: 674, 1945.
- (4) FRAME, E. G., J. A. RUSSELL AND A. E. WILHELMI. J. Biol. Chem. **149**: 255, 1943.
- (5) LEVA, E. J. Biol. Chem. **132**: 487, 1940.
- (6) DARROW, D. C. AND F. L. ENGEL. This Journal **145**: 32, 1945.
- (7) ENGEL, F. L. AND M. G. ENGEL. This Journal **147**: 165, 1947.

THE INFLUENCE OF FEEDING ON THE EFFECTS OF HEPATIC ANOXIA ON THE RESPIRATION OF LIVER SLICES IN VITRO¹

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In fasted eviscerated rats in which the blood supply to the liver is provided only by the hepatic artery, occlusion of the artery for increasing periods of time leads to an increasing depression of the rate of oxygen uptake of the liver tissue. If the hepatic arterial blood supply is restored after a period of anoxia due to arrest of the circulation, the rate of respiration of the liver tissue, taken two hours after blood flow through the liver is restored, increases by an amount inversely proportional to the duration of the preceding period of anoxia (1). These increases in the rate of oxygen uptake of the liver tissue may be correlated with the restored ability of the intact liver, recovering after similar periods of anoxia, to assimilate amino acids from the blood (2). It was suggested that the rate of oxygen uptake of the liver tissue provides a fair estimate of the general level of hepatic function and that the eviscerate rat preparation might be useful for studying certain factors influencing the resistance and recovery of the liver *in vivo* when it is subjected to anoxia uncomplicated by shock due to hemorrhage.

This is a report of further observations upon hepatic resistance and recovery in eviscerated rats. It was found, first, that the rates of respiration of liver tissue from fed animals (on the stock diet of Fox Chow) were less seriously depressed by anoxia due to arrest of the hepatic circulation and that recovery when the blood supply was restored was more complete than in fasted rats. In order to see if these effects were due to one or another of the major components of the diet, the observations were repeated upon groups of rats fasted for 24 hours and then given by stomach tube, one or two hours before operation, glucose, dl-methionine or dl-alanine, or soy bean oil. Sodium chloride solution was given to one group of animals in order to observe, independently, the effects of increased blood flow through the abdominal organs. In two other series of experiments glucose was injected subcutaneously, and dl-methionine was injected intravenously, into fasted eviscerate preparations at the beginning of the two-hour recovery period after 60 minutes of arrest of the hepatic arterial blood supply. Finally, a limited series of observations was made upon a group of fed animals maintained on a low-protein diet.

MATERIALS AND METHODS. Male albino rats of the Sprague-Dawley strain, weighting from 220 to 260 grams, were used. They were kept in the laboratory for at least a week before use and were fed a stock diet of Fox Chow, except in the instance of one group of rats, which were given, for 7 to 12 days before use, a

¹ The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and Yale University.

low-protein diet of the following percentage composition: cornstarch, 62; Crisco, 24; cod liver oil, 2; dried yeast, 8; Osborne and Mendel salt mixture, 4.

All operations were carried out under nembutal anesthesia. The method of preparing eviscerate rats with the blood supply to the liver provided only by the hepatic artery has been described elsewhere (2). The left lobe of the liver was used in each instance to prepare thin slices for the measurement of oxygen uptake in the Warburg apparatus. Samples of about 100 mgm. wet weight were prepared in triplicate by methods outlined in another paper (3). Parallel samples of about 50 mgm. wet weight were dried to constant weight in an oven at 110°C. The percentage dry weight so determined was used to calculate the dry weight of the incubated tissue samples, and the rates of oxygen uptake are, therefore, expressed as cubic millimeters of oxygen per milligram of *initial* dry weight per

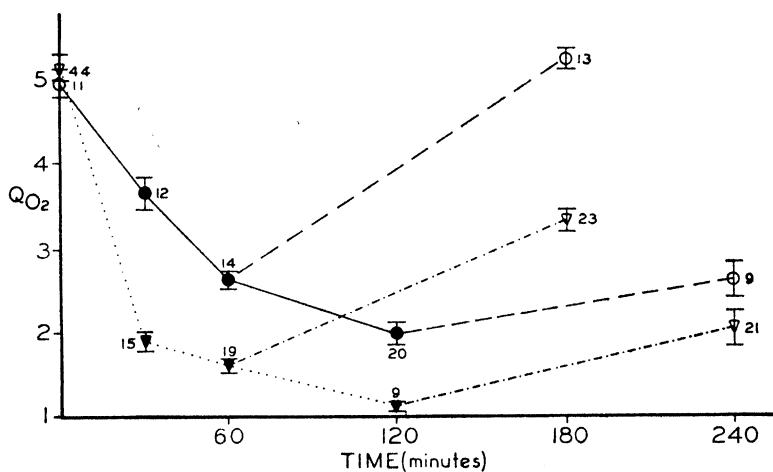


Fig. 1. Effect of hepatic anoxia *in vivo* on the respiration of liver slices from fed (solid and open circles) and fasted (solid and open triangles) rats. The small numbers are the number of observations. The distance between the horizontal bars at each point is twice the standard error.

hour. The tissue slices were suspended in 3 ml. of physiological salt solution buffered with phosphate (0.017 M; pH 7.4), under an atmosphere of 100 per cent oxygen. The oxygen uptake was measured for one hour after an initial 10-minute period to allow temperature equilibration to take place. The time of preparation of the samples was kept as uniform as possible.

RESULTS. The oxygen uptake of samples of liver tissue from fed rats on the stock diet was measured *a*, after interrupting blood flow through the liver by clamping the hepatic artery for 30, 60 or 120 minutes; and *b*, two hours after restoring blood flow through the liver by releasing the hepatic artery after it had been clamped for 60 or 120 minutes; and *c*, immediately after the operation (when the liver had been receiving a purely arterial blood supply for about 5 min.) in order to obtain the initial rate of respiration of the tissue. These observations

are summarized and compared with corresponding observations on fasted rats in figure 1.

The initial rates of respiration are not significantly different in samples from fed and fasted rats. With increasing duration of anoxia, the fall in rate of oxygen uptake is smaller in the livers from fed rats. The order of recovery of the oxygen uptake in two hours after 60 minutes of circulatory arrest is both absolutely and relatively greater in the fed rats. In these animals the rate of respiration returns to the initial level, whereas in fasted rats the oxygen uptake returns to the initial level only after 15 minutes of anoxia (1). This advantage of the fed animals in recovery is not retained after 120 minutes of anoxia.

These observations are compared with those made upon fed rats on the low-protein diet in figure 2. The principal effect of the diet is to depress the initial

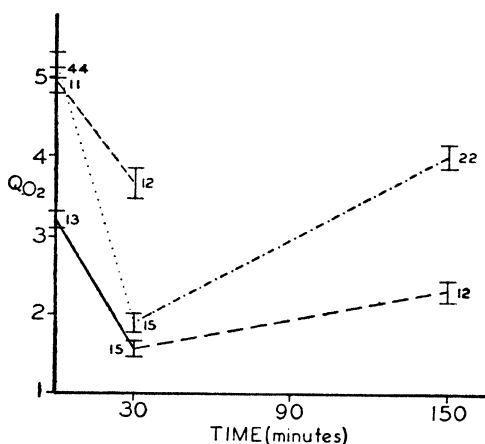


Fig. 2. Effects of hepatic anoxia *in vivo* on the respiration of liver slices from fed rats on a low protein diet. Solid line followed by heavy dashed line: fed low protein; dotted line followed by dot-and-dashed line: fasted normal; light dashed line: fed normal. The small numbers are the number of observations. The distance between the horizontal bars at each point is twice the standard error.

rate of respiration of the liver tissue to about 60 per cent of the normal rate. In terms of this initial rate, the effect of 30 minutes of anoxia and the order of magnitude of recovery compare favorably with the effects (relative to the normal initial rate) seen in the livers of fed and fasted rats on the stock diet. In terms of the absolute rate of oxygen uptake, however, the respiration of the liver tissue from rats on the low-protein diet is poor, and its reserve after only 30 minutes of anoxia is small.

Since the livers of fed rats, both on the stock diet and on the low-protein diet, contain 4 to 7 per cent of carbohydrate, and the active tissue is, therefore, "diluted" by the inert, stored polysaccharide, it may be argued, as Fuhrman and Field (4) have done, that the rates of respiration calculated on the basis of the initial dry weight of the tissue are under-estimated and are not properly comparable with those of fasted rat livers of low glycogen content. This objection

may be met in part by recalculating the rates of oxygen uptake in terms of the final dry weight of the tissue, since during incubation in phosphate-buffered saline media, most of the glycogen in rat liver slices is broken down to free glucose (5). The recalculated data are presented in table 1; they merely emphasize the relative incompetence of the liver tissue from rats on the low-protein diet.

The greater resistance of the livers of fed rats to anoxia *in vivo* is similar to that exhibited by liver slices from fed rats after incubation under nitrogen *in vitro* (3, 6). This may be due in part to the higher glycogen content of the livers of fed rats and to the consequent greater accumulation of lactic acid during anaero-

TABLE 1

Rates of oxygen uptake of liver slices from fasted and fed rats on a stock diet and from fed rats on a low-protein diet, before and after anoxia and recovery, in vivo

	OXYGEN UPTAKE (CU. MM. PER MGM. OF FINAL DRY WEIGHT PER HOUR)		
	Initial	Anoxia (30 min.)	Recovery (2 hrs.)
Fasted (stock diet).....	7.38	3.28	6.11
Fed (stock diet).....	8.50	6.41	(8.50)*
Fed (low-protein diet).....	6.00	3.26	4.44

(Final dry weight = weight of slices removed at end of incubation period, rinsed in distilled water, and dried to constant weight at 110°C.)

* Recovery assumed to be complete, since, after 60 minutes of anoxia, the liver respiration recovers completely.

TABLE 2

Losses in weight of liver slices during incubation before and after anoxia and recovery

	WEIGHT LOSS DURING INCUBATION (PER CENT OF INITIAL DRY WEIGHT)		
	Initial	Anoxia (30 min.)	Recovery (2 hrs.)
Fasted (stock diet).....	30.2	42.2	34.5
Fed (stock diet).....	41.7	43.1	
		43.5*	30.8**
Fed (low-protein diet).....	46.7	52.1	47.8

* Anoxia: 60 minutes.

** Recovery after 60 minutes of anoxia.

biosis (6). The presence of glucose (200 mgm. per cent) in the medium has, however, no effect on the difference in resistance to anoxia between liver slices from fed and fasted rats (3). Liver slices may lose from 30 to more than 50 per cent of their dry weight to the medium during incubation (7, 8, 9), and the lost substances may play a part in supporting respiration *in vitro*, or their loss may be related to a failure of energy-yielding systems to maintain the integrity of the tissue. In table 2 it may be seen that the loss in weight of liver slices from fed rats on the stock diet is only slightly increased by 30 or 60 minutes of anoxia, whereas liver slices from fasted rats, as well as those from low-protein-fed rats

lose much more substance during incubation after 30 minutes of hepatic anoxia *in vivo*. The larger initial weight loss of liver slices from fed rats is due to their content of glycogen, which breaks down to glucose during incubation. It will be noted that glycogenolysis can account only in part for the total loss in weight during incubation. After two hours of recovery *in vivo* from a previous period of anoxia, the weight losses return toward the initial value. The order of recovery in this respect runs parallel to the order of recovery of the oxygen uptakes seen in figures 1 and 2 and table 1.

The effects of some of the individual components of the diet on hepatic resistance to and recovery from anoxia were studied by giving to 24-hour fasted rats, by stomach tube, 1 to 2 hours before operation, one of the following: 1, 5 ml. of 20 per cent glucose; 2, 75 mgm. (in 5 ml. of water) of dl-methionine; 3, 45 mgm. (in 5 ml. of water) of dl-alanine; 4, 1.5 to 3 ml. of soy bean oil; 5, 5 ml. of 2 per cent sodium chloride solution. In the glucose-, methionine-, and sodium-chloride-treated rats, liver tissue respiration was measured both after 60 minutes of arrest of the hepatic blood supply and after 2 hours of recovery from 60 minutes of anoxia. In the other groups, liver respiration was measured only after recovery from 60 minutes of anoxia. In addition, the effects of glucose and of dl-methionine on recovery after 60 minutes of anoxia were studied by injecting, just before the release of the hepatic artery, *a*, 1 ml. of 15 per cent glucose, in 4 equal doses, subcutaneously; or *b*, 30 mgm. of dl-methionine, into the femoral vein in 4 doses of 0.25 ml. each at 0, 0.5, 1.0, and 1.5 hours, or in 2 doses of 0.5 ml. each at 0 and 1 hour after release of the hepatic artery; or *c*, 30 mgm. of dl-methionine into the stump of the portal vein.

The results of these experiments are summarized in figure 3, in which the values for both fed and fasted rats are presented for comparison. Pretreatment of fasted rats with glucose results in a significantly larger oxygen uptake of tissue slices from livers subjected to 60 minutes of anoxia (column C) than that of liver slices from untreated fasted rats (column A'), but the respiration is still significantly less than that of liver slices from fed rats (column B'). Pretreatment with dl-methionine results in a slightly higher oxygen uptake (column D), and sodium chloride treatment (column E) is without effect. One effect of feeding is a large increase in the rate and volume of blood flow through the abdominal organs, and this might be accompanied by an increase in the metabolic activities of the liver, as well as of the gastrointestinal tract. The feeding of sodium chloride solution stimulates visceral circulation and metabolism (10), and the effects of this on the behavior of the liver may, therefore, be observed independently of the effects of administered foodstuffs. It may be noted that, although the effects of sodium chloride and of dl-methionine on the liver respiration after anoxia were absent or small, the extra loss in weight during incubation seen in liver slices from fasted rats after hepatic anoxia (table 2) is not seen in liver slices from the methionine- or sodium-chloride-treated rats. The suggestion that the integrity of the tissue is better maintained in these two groups of animals is borne out by the observations on recovery of the liver respiration after anoxia (columns D' and E').

In two hours after blood flow through the liver is restored, the rate of oxygen uptake of the liver tissue from rats pre-treated with glucose, dl-methionine, dl-alanine, and sodium chloride (columns C', D', E', and I) increases to nearly normal levels and is in each instance significantly greater than the rate of oxygen uptake of liver slices from fasted rats (column A''). The injection of glucose or of dl-methionine during the recovery period is without effect (columns F, G, and H). Pre-treatment with fat (column J) is also without effect on the recovery of liver

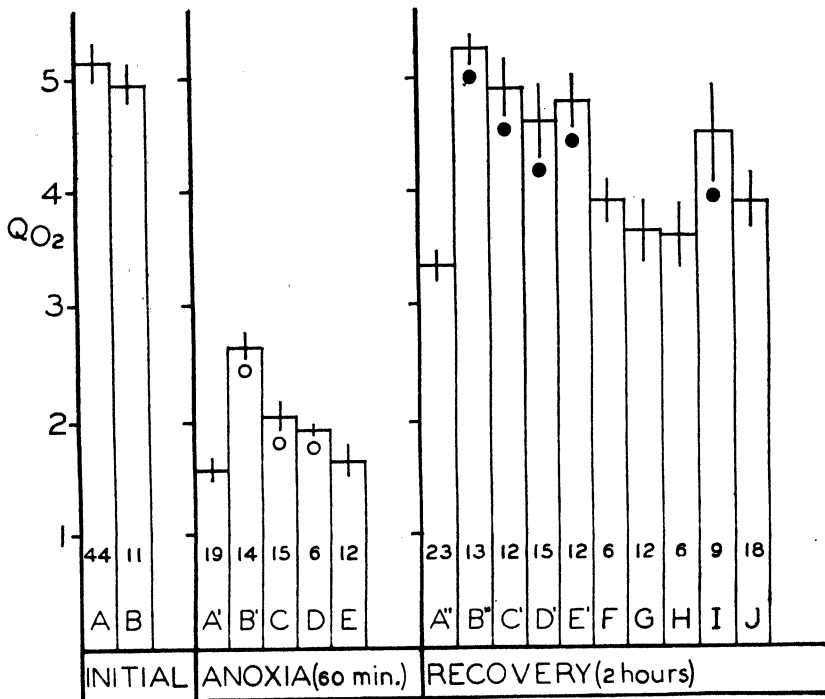


Fig. 3. The effects of hepatic anoxia *in vivo* on the respiration of liver slices from fasted rats (columns A, A' and A''), fed rats (columns B, B' and B'') and fasted rats given by stomach tube, glucose (columns C and C'), dl-methionine (columns D and D'), sodium chloride solution (columns E and E'), dl-alanine (column I), or soy bean oil (column J); or given, during the recovery period, glucose, subcutaneously (column F), or dl-methionine, intravenously into the femoral vein (column G) or into the portal vein (column H). The number of observations is given in each column. The vertical bars are twice the standard error in length. Hollow circles indicate values significantly greater than that of column A'; solid circles indicate values significantly greater than that of column A''.

respiration after anoxia, although, as in the other feeding experiments, there was a pronounced increase in blood flow through the abdominal organs for 1 to 2 hours before the evisceration was done.

DISCUSSION. The foregoing observations indicate that at least two factors in hepatic resistance to and recovery from anoxia due to arrest of the blood supply are 1, the availability of metabolizable substrate (glucose or glycogen) during the period of anaerobiosis, and 2, the rate and volume of blood flow through the

abdominal organs during the period before the hepatic blood supply is cut off.

During anaerobiosis a supply of energy from glycolysis may secure the integrity of the respiratory enzymes and co-enzymes and may also prevent the accumulation of undesirable metabolites. In fasted rats pretreated with glucose or with dl-methionine, liver glycogen may be expected to reach levels of one per cent or more in the 1 to 2 hour period before operation, and this accumulation of substrate for glycolysis may be related to the moderate increase in the respiration of the liver tissue, over that of tissue slices from untreated fasted rats, or from sodium-chloride-treated rats seen after 60 minutes of anoxia. Neither of these treatments preserves the rate of oxygen uptake at the levels attained by liver tissue from fed rats, so that an ample supply of carbohydrate is not the only factor concerned in the resistance of the liver to anoxia in the fed animal. In fed and fasted rats the relative proportions of the major foodstuffs being catabolized by the liver differ widely. In liver slices from fasted rats the R. Q. of the order of 0.5 and the rate of formation of ketone bodies indicate that fat is the chief, if not the only, substrate being oxidized. The accumulation of free fatty acids in the liver during anoxia is likely to be both greater and more rapid when fat is being actively catabolized than when, as in the fed animal, fat may be a minor substrate for liver tissue oxidations. If, as Lehninger's recent observations suggest (11), free fatty acids inhibit liver respiration, then this effect may be expected to be greatest in liver tissue from fasted rats after anoxia, and marked, but less great in liver tissue from animals in which the hepatic metabolic pattern is intermediate between the fed and the fasted state, that is, during the assimilation of glucose or a glucose-yielding substrate. The relation between the level of free fatty acids in the liver and the rate of liver respiration, after anoxia and during shock after hemorrhage, requires more direct experimental investigation.

The effect of pretreatment with sodium chloride on the recovery of liver respiration after anoxia may be interpreted as an effect of the associated increase in rate and volume of blood flow through the abdominal organs over that characteristic of the fasted state. This effect, which is independent of administered substrate, must take place before the experiment proper, in which the rat is eviscerated, is carried out. It may comprise one or both of two factors: 1, that a period of brisk circulation of blood through the liver may open up freely the connections between the arterial and the portal sides of the hepatic vascular system and thus facilitate the prompt return of blood flow throughout the liver, from the arterial side only, after temporary arrest of the circulation; and 2, that an increase in the rate of metabolic processes in the liver may accompany the increase in blood flow and may be attended by the mobilization of essential substances to the liver from other tissues.

If the first factor plays a large part in determining the recovery of liver respiration, then most of these observations depend upon a peculiar property of the eviscerate rat preparation: that the ease of establishing blood supply to the liver from the arterial side only is a function of the previous state of activity of the hepatic vascular system. This may be doubted for the following reasons. First, it is improbable that a purely mechanical state—the patency of the arterio-

venous connections—would persist during a 60-minute period of circulatory arrest. Second, in normal fasted rats (1) and in fasted adrenalectomized rats maintained on desoxycorticosterone (12), the sudden change from a mixed to a purely arterial blood supply is not followed (in the first or second hour) by a significant decrease in the rate of the liver tissue respiration, or (in normal fasted rats) by a temporary failure of the liver to remove amino acids from the blood (2). If in these fasted animals the arteriovenous connections are not as freely established as in fed rats, the period of relative anoxia before the blood supply from the arterial side becomes fully effective cannot be long. Finally, it is observed in most eviscerate preparations, both fed and fasted, that when the hepatic artery is released after a period of anoxia, the port-wine color of the liver due to reduced hemoglobin changes within a few minutes to its normal red-brown tone. Differences in the rate of establishment of blood flow throughout the liver do not, therefore, seem to play a major part in determining the differences in recovery of liver respiration observed in fed and fasted rats.

The second factor—the metabolic consequences of a period of increased circulation through the abdominal organs—may be of considerable importance in hepatic resistance and recovery after stress. The observation of Brodie, Cullis and Halliburton (10) that the administration merely of salt solution (to cats) is followed by one- to two-fold increases in intestinal blood flow, oxygen consumption, and carbon dioxide production, indicates that the metabolic activities of the gastrointestinal system are stimulated by feeding. The liver, as an essential digestive organ normally receiving most of its blood and oxygen supply from the venous outflow of the gastrointestinal tract, may be expected to participate in this metabolic stimulation. This means that there is a quickening and probably a wide extension of the many related intermediary reactions involved in principal digestive function of the liver—the assimilation of nutrients, that there is an increased rate of energy production, and that there may be an increase in the level of readily available chemical energy (of high-energy phosphate) in anticipation of the digestive load. In these circumstances the stress of a period of anoxia can be met with a greater supply of energy, 1, to facilitate glycolysis (since this process requires energy in its initial stages, for the phosphorylation of glucose or of fructose-6-phosphate) and thus to assure a continuing, if limited, supply of energy during anoxia, and 2, to help maintain in their active forms many of the co-factors necessary for normal tissue respiration, which are damaged or destroyed in anoxic tissues (13, 14).

In this connection, another consequence of the transition from the fasting to the fed state may be considered. Supplee and his co-workers (15) have found that during the digestion and assimilation of food in young white rats, there is a mobilization of riboflavin from other tissues to the liver, and that the increase in liver riboflavin concentration may persist for some hours after feeding. They did not extend their observations to other vitamins, nor did they examine the possibility, by feeding salt solution rather than the basal diet, that the mobilization of riboflavin (and other essential substances) to the liver can take place

following the stimulation of gastrointestinal activity in the absence of administered nutrients, as a part of the general preparatory reactions of the system for the task of digestion and assimilation. Since riboflavin is an essential component of a large group of respiratory enzymes, an increased concentration of the vitamin in the liver is likely to improve the chances of recovery of liver respiration after a period of anoxia.

In the experiments in which fat was fed, recovery of the liver respiration after anoxia was smaller than in any of the other feeding experiments. It may be that the advantages gained by the stimulation of liver circulation and metabolism were outweighed by the consequences of active fat catabolism in the liver which were outlined in the first part of this discussion. In this instance, however, the estimate of recovery may be too low because of the nature of the test method, since in the course of preparing the tissue slices a period of relative anoxia of the tissue, during which free fatty acids might accumulate, is unavoidable. Some evidence of this is suggested by the facts that in this series of experiments the variations between animals and between individual samples were somewhat larger than usual, and that the rates of oxygen uptake of the tissue samples in a single experiment were often successively lower, in the order in which they were prepared and distributed to the reaction vessels.

These experiments were undertaken as a part of a study of damage and repair of the liver in shock after hemorrhage and other severe stresses. The evidence indicates that in the rat the intact liver, served only by the hepatic artery, can recover to a considerable extent from the severe stress of 60 minutes of anoxia due to an interrupted blood supply. The provision of extra substrate (glucose or dl-methionine) after a period of anoxia does not accelerate recovery, but these measures are undoubtedly too simple to meet all the requirements for repair. In the treatment of shock, the restoration of normal blood volume and oxygen-carrying capacity is sometimes insufficient to bring about recovery. In these circumstances, as Fine and his colleagues (16) have shown, viviperfusion of the liver of an animal in shock with arterial blood from another animal may bring about recovery. This suggests that the restoration of normal blood volume and oxygen-carrying capacity is not always followed by an adequate increase in the rate and volume of blood flow through the abdominal organs. The liver, which in the intact animal receives most of its blood and oxygen supply through the portal system, may, therefore, not be able to exercise its native capacities for recovery. The effects of preliminary feeding, even of salt solution, on the recovery of liver respiration after anoxia suggest that in the treatment of shock some additional means of stimulating normal gastrointestinal activity might not only assure an adequate blood and oxygen supply to the liver, but might also bring about the mobilization of essential substances to the liver which can aid in restoring normal function. In this connection the reported beneficial effects of Spasokutotski's (17) method of introducing a rich food mixture into the intestine in the course of surgical repair of penetrating abdominal wounds, are of great interest.

SUMMARY

In fed rats the resistance of liver respiration to anoxia due to arrest of the blood supply is greater, and recovery during a standard period of restored circulation is more complete, than in fasted rats.

The administration to fasted rats, by stomach tube, 1 to 2 hours before operation (evisceration), of glucose, dl-methionine, dl-alanine, soy bean oil, or sodium chloride solution, is followed by a moderate increase in the resistance of liver respiration to anoxia only after glucose, and by nearly complete recovery of the liver respiration to levels characteristic of fed rats in every instance except after feeding fat. The administration of glucose (subcutaneously) or of dl-methionine (intravenously, into the femoral vein or into the stump of the portal vein) during the recovery period only, does not accelerate the repair of the liver respiration.

Some of the factors involved in the damage and repair of the liver after anoxia are discussed and are referred to the related problem of the restoration of normal liver function after shock.

REFERENCES

- (1) WILHELMI, A. E., J. A. RUSSELL, F. L. ENGEL AND C. N. H. LONG. *This Journal* **144**: 669, 1945.
- (2) ENGEL, F. L., H. C. HARRISON AND C. N. H. LONG. *J. Exper. Med.* **79**: 9, 1944.
- (3) RUSSELL, J. A., C. N. H. LONG AND A. E. WILHELMI. *J. Exper. Med.* **79**: 23, 1944.
- (4) FUHRMAN, F. A. AND J. FIELD, 2ND. *Arch. Biochem.* **6**: 337, 1945.
- (5) WILHELMI, A. E. AND J. A. RUSSELL. Unpublished observations.
- (6) CRAIG, F. N. *J. Biol. Chem.* **150**: 209, 1943.
- (7) ELLIOT, K. A. C., M. E. GREIG AND M. P. BENOY. *Biochem. J.* **31**: 1003, 1937.
- (8) BACH, S. J. *Biochem. J.* **38**: 156, 1944.
- (9) WILHELMI, A. E. Unpublished observations.
- (10) BRODIE, T. G., W. C. CULLIS AND W. D. HALLIBURTON. *J. Physiol.* **40**: 173, 1910.
- (11) LEHNINGER, A. L. *J. Biol. Chem.* **157**: 363, 1945.
- (12) WILHELMI, A. E., M. G. ENGEL AND C. N. H. LONG. Unpublished observations.
- (13) GREIG, M. E. AND W. M. GOVIER. *J. Pharmacol. and Exper. Therap.* **79**: 169, 1943.
- (14) GREIG, M. E. *J. Pharmacol. and Exper. Therap.* **81**: 164, 1944.
- (15) SUPPLEE, G. C., O. G. JENSEN, R. C. BENDER AND O. J. KOHLENBERG. *J. Biol. Chem.* **144**: 79, 1942.
- (16) FINE, J., H. A. FRANK AND A. M. SELIGMAN. *Ann. Surg.*, **122**: 652, 1945.
- (17) PANIKOV, P. A. *Am. Rev. Soviet Med.* **1**: 32, 1943-44.

ON THE DETERMINATION OF THE PHYSIOLOGICALLY EFFECTIVE PRESSURES OF OXYGEN AND CARBON DIOXIDE IN ALVEOLAR AIR¹

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Although studies of the composition of alveolar air have been invaluable to the advance of respiratory physiology a precise definition of alveolar gas pressures and the accurate sampling of alveolar air have proved elusive goals (1). Owing to the cyclic nature of the ventilatory process the partial pressures of O₂ and CO₂ in the alveoli are changing continually (time factor) (9), and, owing to inequalities in intrapulmonary ventilation and circulation, the partial pressures in different parts of the lungs may differ significantly (space factor) (10, 15). Therefore, the concept that a single value represents the partial pressure of an alveolar gas requires the assumption that the spot sample of alveolar air be representative with respect to both time and space.

There are cogent reasons for believing that the two principal methods for sampling alveolar air (the single complete expiration method of Haldane and Priestley and the fractional sampling technic of Sonne and Nielsen) do not guarantee that the sample always is, in fact, representative. Neither method insures that the sample contains proportional contributions of alveolar air from all portions of the lung (space error), nor that the sample obtained has not lost O₂ and gained CO₂ during the brief period of stasis within the alveoli (time error). For example, the partial pressures of O₂ and CO₂ in samples of alveolar air obtained by the Haldane-Priestley technic vary with respect to the timing of the expiratory effort (end-inspiration or end-expiration) (5, 6). And again, fractional sampling of the alveolar air by the Sonne-Nielsen technic has yielded evidence that successive samples of alveolar air taken at different stages during expiration vary appreciably with respect to gaseous composition (14).

Despite these limitations many fundamental contributions to an understanding of respiratory mechanisms have come from studies of the composition of alveolar air at rest (4, 7). However, during even moderate exercise the rate of evolution of CO₂ into and the escape of O₂ out of the alveoli may be increased tenfold or more so that the slight delay necessary to expel the alveolar sample is sufficient to permit radical changes to develop. This failure of the direct sampling technics to provide reliable data during exercise has led us to measure alveolar gas pressures by an indirect method now to be described.

The indirect measurement of alveolar gas pressures. Indirectly determined alveolar CO₂ and O₂ pressures are calculated from the arterial pCO₂ and the

¹The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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$p\text{CO}_2$ and $p\text{O}_2$ of the inspired and expired air. The significance of these calculated alveolar pressures depends on the interrelations of the various factors entering into the calculation and will be considered below in detail. We may state at this point, however, that the goal of the indirect method is to determine the physiologically effective partial pressures of CO_2 and O_2 in the alveoli. The effective alveolar pressures may be defined precisely as those pressures which, if present continually and uniformly in all functioning alveoli, would permit the exchange of CO_2 and O_2 between the alveoli and the blood, during a series of ventilatory cycles, in amounts exactly equal to the gas exchange as measured from analyses of the inspired and expired air.

In order to discuss certain differences between ideal direct and indirect samples we shall present first our concept of an ideal direct sample, as furnished by the most frequently used technic, the Haldane-Priestley. In this technic a complete forcible expiration is performed which ideally would squeeze all of the alveolar air from the lung. If this goal were attained the ideal direct sample would contain contributions from each alveolus in proportion to the *volume* of air which it contained. By contrast, the indirect method is based, in the case of oxygen, upon the concept that in an ideal alveolar sample the contribution of each alveolus is in proportion to its *ventilation*. There probably would be slight differences between what we shall call the *average* (direct) values and the *effective* (indirect) values, even under ideal conditions, due primarily to inequalities in the ventilation-perfusion ratio in different parts of the lungs.

Estimation of effective alveolar $p\text{CO}_2$. The estimation of effective alveolar $p\text{CO}_2$ is based on the assumption that effective alveolar $p\text{CO}_2$ equals arterial $p\text{CO}_2$ (3). The arterial $p\text{CO}_2$ is thus considered to equal the mean of the entire range of alveolar CO_2 pressures existing in different parts of the lung and at different moments in the ventilatory cycle. The validity of this assumption depends on the magnitude of two unfavorable factors: *a*, the tendency of the arterial $p\text{CO}_2$ to be higher than alveolar $p\text{CO}_2$ because of the resistance offered by the "pulmonary membrane"; and *b*, the tendency of the peripheral arterial $p\text{CO}_2$ to exceed alveolar $p\text{CO}_2$ owing to the admixture of venous blood with a higher CO_2 tension. The sources of such venous admixture might be 1, blood which has passed through inadequately ventilated alveoli; 2, the bronchial circulation; 3, the anterior cardiac and Thebesian veins of the left heart, and 4, such venous-to-arterial shunts as may exist within the lesser circulation.

The resistance of the pulmonary membrane to the escape of CO_2 may be dismissed as an insignificant factor because of the rapid diffusion of CO_2 through tissues, estimated by Krogh to be 30 times as great as that of O_2 (11). The amount of admixture of non-arterialised blood arising from the various sources listed above (factor *b*) may be estimated by expressing the joint effect as though there had been added a certain volume of mixed venous blood to completely arterialised blood. For example, let us consider the extreme situation where each 80 parts of fully arterialised blood will have 20 parts of mixed venous blood added to them (from the above sources) before they are ejected into the peripheral arterial tree (2). What change in $p\text{CO}_2$ will result? If a mixture is made of

bloods of the following composition in the given proportions we can calculate by means of the Henderson-Hasselbalch equation what the characteristics of the resulting mixture will be:

	<i>parts</i>	<i>pCO₂</i>	<i>pH_s</i>	<i>[CO₂] vols. p. c.</i>
Arterial.....	80	38	7.400	53.6
Venous.....	20	45	7.371	59.4

The CO₂ content of the mixture is calculated thus: $(0.8 \times 53.6) + (0.2 \times 59.4) = 54.7$ vols. per cent which, at a pH_s of 7.394, will exert a pCO₂ of 39.5 mm. Hg. Therefore, the addition of 1 part of venous blood to 4 parts of arterial blood will raise the pCO₂ of the peripheral arterial blood only 1.5 mm. Hg. As will be seen below an error of 1.5 mm. Hg in determining the effective alveolar pCO₂ will introduce an error in the estimation of alveolar pO₂ which will not exceed 2 mm. Hg.

It need hardly be emphasized that the presence of a congenital cardiac anomaly which deflects enormous quantities of venous blood directly into the arterial tree will introduce a larger divergence of arterial from alveolar pCO₂.

Estimation of effective alveolar pO₂. Because a significant tension gradient may exist between alveolar air and arterial blood (3), it cannot be assumed, as in the case of CO₂, that the effective alveolar pO₂ and the arterial pO₂ are equal. However, given a known value for effective alveolar pCO₂ and a known relationship between the effective alveolar pCO₂ and pO₂ one could calculate the effective alveolar pO₂.

What then is the relationship between the effective alveolar pCO₂ and pO₂, i.e., the effective alveolar RQ? Let us consider first the expired air, which is simply a mixture of alveolar air and dead space air. The RQ of the expired air is determined solely by the alveolar component since the dead space air is merely a diluent (1,8). Furthermore, the alveolar component of the expired air contains contributions from each alveolus in proportion to its ventilation. Accordingly, the alveolar component of the expired air is the effective alveolar air, and the expired air RQ is the effective alveolar RQ.³ The required relationship between effective alveolar pCO₂ and pO₂ is thus provided by the expired air RQ.

The effective alveolar pO₂ can be calculated then from determinations of the arterial pCO₂ and the RQ of the expired air without sampling the alveolar air directly. The equation for the calculation is derived from the accepted alveolar equation as follows:

$$\text{alveolar RQ} = \frac{\text{alveolar pCO}_2}{\text{tracheal pO}_2^4 \times \left(\frac{\% \text{ expired N}_2}{\% \text{ inspired N}_2} \right)^5 - \text{alveolar pO}_2} \quad (1)$$

³ Ferguson and Dugal recently showed that for normal resting subjects during anoxia (when the time factor is much reduced) the alveolar RQ determined from the direct sample closely approximated the expired air RQ, indicating that under these conditions the average and the effective alveolar pressures are very nearly identical (6).

⁴ 37°C., saturated, ambient pressure.

⁵ The tracheal pO₂, when multiplied by this ratio, is expressed in terms of expired air.

effective alveolar RQ =

$$\frac{\text{effective alveolar } p\text{CO}_2}{\text{tracheal } p\text{O}_2 \times \left(\frac{\% \text{ expired } \text{N}_2}{\% \text{ inspired } \text{N}_2} \right) - \text{effective alveolar } p\text{O}_2} \quad (1a)$$

$$\text{effective alveolar } p\text{CO}_2 = \text{arterial } p\text{CO}_2 \quad (2)$$

$$\text{effective alveolar RQ} = \text{expired air RQ} \quad (3)$$

Substituting (2) and (3) in equation (1a):

expired air RQ =

$$\frac{\text{arterial } p\text{CO}_2}{\text{tracheal } p\text{O}_2 \times \left(\frac{\% \text{ expired } \text{N}_2}{\% \text{ inspired } \text{N}_2} \right) - \text{effective alveolar } p\text{O}_2} \quad (4)$$

and transposing:

effective alveolar $p\text{O}_2$ =

$$\text{tracheal } p\text{O}_2 \times \left(\frac{\% \text{ expired } \text{N}_2}{\% \text{ inspired } \text{N}_2} \right) - \frac{\text{arterial } p\text{CO}_2}{\text{expired air RQ}} \quad (4a)$$

It will be apparent that for the purpose of constructing the equations above the effective alveolar $p\text{CO}_2$ and the effective alveolar $p\text{O}_2$ are not derived in an entirely parallel manner. The use of the arterial $p\text{CO}_2$ as a measure of the effective alveolar $p\text{CO}_2$ weights the contribution of each alveolus in proportion to its perfusion with blood, while the use of the expired air RQ for the calculation of the effective alveolar $p\text{O}_2$ weights the contribution of each alveolus in proportion to its ventilation. As shown above, however, even as gross a distortion of the ventilation-perfusion ratio as a 20 per cent venous-to-arterial shunt will lead to a minimal change in the determined value of alveolar $p\text{CO}_2$. For practical purposes, then, the indirectly determined alveolar $p\text{CO}_2$ may be considered to be effective in the same sense that the indirect alveolar $p\text{O}_2$ is effective.

The errors introduced into the calculation of effective alveolar $p\text{O}_2$ as a result of technical errors in the analysis of the respired gases will be less than 0.5 mm. Hg. The only source of significant error is the determination of the arterial $p\text{CO}_2$. This error rarely exceeds ± 3 mm. Hg and it will affect the calculated alveolar $p\text{O}_2$ accordingly: if the RQ is 1.0 the error in effective alveolar $p\text{O}_2$ will be equal and opposite to the error in arterial $p\text{CO}_2$; the maximum error will occur when the RQ is low but will not exceed 4 mm. Hg.

METHODS. *Experimental determination of effective alveolar pressures.* In order to estimate the effective alveolar pressures, the following determinations were made on five male subjects during 31 experiments:

1. *The $p\text{CO}_2$ of the arterial blood.* The sample was withdrawn at a slow, steady rate for a period of one minute through an indwelling needle introduced earlier into the brachial artery through an area of local anesthesia. The $p\text{CO}_2$ was determined by the bubble method of Riley, Proemmel and Franke (13).

2. *The CO_2 and O_2 pressures of the expired air.* The expired air was collected by standard technics in a Douglas bag for a period of one minute. The collection period for expired air began 5 to 10 seconds before the sampling of arterial blood was begun and was ended a similar brief period before the arterial sampling was completed. Gas analyses were performed in the Haldane-Henderson apparatus. These determinations furnished the data for calculating the expired air RQ.

3. *The CO_2 and O_2 pressures of the inspired air.*

The effective alveolar pO_2 was calculated by substituting these determined values in equation 4a.

Comparison of effective alveolar pressures with the pressures in Haldane-Priestley samples of alveolar air. The direct samples of alveolar air were obtained by the Boothby modification of the Haldane-Priestley technic (a sharp deep expiration at the end of a normal inspiration) (4) after the conclusion of the minute period during which the arterial blood and the expired air were collected. The subjects were trained carefully until consistent samples of alveolar air were obtained repeatedly. The experiments at rest were performed with the subject either lying supine or standing quietly but without any standardized period of rest. The exercise experiments were performed on a motor-driven treadmill inclined 5 degrees (8.8 per cent grade) moving at 4.8 Km./hr. (3 m.p.h.). The exercise was continued for 5 to 8 minutes, or until the minute ventilation had become constant, before samples were obtained. This grade of work required an oxygen consumption which averaged 1.5 l./m. Experiments were performed while subjects were breathing room air or oxygen-nitrogen mixtures designed to produce moderate anoxia.

RESULTS. The comparative data obtained from 31 experiments are presented in table 1. The differences at rest between effective alveolar pressures and Haldane-Priestley alveolar pressures are, in general, not great. During exercise, however, the accumulation of CO_2 and the depletion of O_2 in the direct alveolar samples was so large that the direct samples yielded values which differed widely from the effective pressures determined by the indirect method.

DISCUSSION. The indirect method for determining alveolar CO_2 and O_2 pressures was developed primarily to circumvent difficulties in the direct methods which are related to the space and time factors. By using the pCO_2 of the arterial blood as a measure of effective alveolar pCO_2 , the blood becomes a physiological integrator of the CO_2 pressures existing in all parts of the lung which are perfused, and errors resulting from the space factor thus are minimised. Likewise, the arterial blood integrates the normal variations in alveolar pCO_2 which occur from moment to moment during the ventilatory cycle, and errors resulting from the time factor are eliminated. The arterial blood can be used in this manner in the case of CO_2 because the blood in the alveolar capillary reaches virtual gaseous equilibrium with the alveolar air.

The experimental finding that the pCO_2 of the Haldane-Priestley alveolar sample is higher than that of arterial blood sampled during the preceding minute probably results from the effect of the time factor which enters into alveolar sampling by the Haldane-Priestley technic: i.e., during the time required to give

TABLE 1

Comparison of effective alveolar pressures (indirect) and Haldane-Priestley alveolar pressures (direct)

SUBJECT	LABORATORY ALTITUDE: 9 FEET			BAROMETRIC PRESSURES: 765-775 MM. HG		
	Effective	H-P	Δ pCO ₂	Effective	H-P	Δ pO ₂
Rest						
Ki	pCO ₂ mm. Hg			pO ₂ mm. Hg		
	39	41	+2	107	95	-12
	36	40	+4	105	105	0
	35	40	+5	102	100	-2
	38	42	+4	102	85	-17
	35	40	+5	105	95	-10
	38	43	+5	71	54	-17
	37	41	+4	63	55	-8
Ko	38	45	+7	103	99	-4
	35	41	+6	107	100	-7
	32	43	+11	111	95	-16
	32	40	+8	70	59	-11
Li	29	38	+9	71	59	-12
	36	40	+4	106	99	-7
	36	40	+4	106	98	-8
	34	35	+1	112	110	-2
Ri	18*	17	-1	87	80	-7
	37	36	-1	58	58	0
	33	38	+5	58	51	-7
Ro	43	42	-1	98	98	0
	35	42	+7	56	46	-10
Average =			+4.4 (-1 to +11)		-7.9 (0 to -17)	
Exercise						
Ki	39	50	+11	108	89	-19
	43	50	+7	103	89	-14
	41	48	+7	63	54	-9
	37	44	+7	57	43	-14
Ko	32	55	+23	115	80	-35
	30	47	+17	77	54	-23
Li	31	46	+15	117	98	-19
Ri	35	44	+9	111	99	-12
	27	40	+13	73	55	-18
Ro	29	40	+11	66	50	-16
	38	58	+20	111	86	-25
Average =			+12.7 (+7 to +23)		-18.5 (-9 to -35)	

* Voluntary hyperventilation.

the alveolar sample, CO₂ continues to be given off from the blood so that the pCO₂ of the alveolar sample is higher than the average level during the relatively steady state before sampling. It is manifestly impossible, on the basis of the

diffusion theory, for the alveolar $p\text{CO}_2$ to be in fact higher than the arterial $p\text{CO}_2$. Error in the Haldane-Priestley sample due to the time factor is increased during exercise not because the time required to give the sample is prolonged but because the rate at which CO_2 is discharged into the alveoli may be increased some ten times. The difference between directly and indirectly determined alveolar $p\text{CO}_2$'s varies from sample to sample and from subject to subject (table 1), owing in part to slight differences in the technic of giving the Haldane-Priestley sample. We should not expect, therefore, a constant high correlation between results by the two methods.

Experimentally, the $p\text{O}_2$ of the Haldane-Priestley sample is lower than the calculated effective alveolar $p\text{O}_2$ because, during the time required to expel the Haldane-Priestley sample, the alveolar $p\text{O}_2$ shifts from the tension normally prevailing toward that obtaining in the mixed venous blood.

It must be emphasized that the differences between the directly and indirectly determined alveolar pressures are due for the most part to the time factor and only in small part to the space factor. Errors in the Haldane-Priestley determinations due to the time factor appear to be significant at rest and very large during exercise and these errors cannot be corrected with accuracy because they are related to slight individual differences in the technic by which the subject gives the sample. These errors, we believe, are obviated by the indirect method.

The use of the expired air RQ in the indirect determination of effective alveolar pressures imposes certain limitations on the experimental application of the indirect method. The measurement of the expired air RQ requires the collection of expired air during a series of ventilatory cycles; if during this period the RQ is changing rapidly (e.g., the first few minutes of exercise or hyperventilation) the calculated effective alveolar pressures will represent a mean of the extremes rather than any instantaneous level. Likewise, if the ventilatory pattern is grossly irregular a similar situation obtains. However, in either instance the effective alveolar $p\text{CO}_2$ and $p\text{O}_2$ as determined are functionally significant values, provided that the periods for collection of the expired air and of the arterial blood are equal and coincident.

The advantages of the indirect method may be summarized as follows: *a*, the effective alveolar pressures represent a functional rather than a volumetric integration of the entire range of pressures existing throughout the lung (space factor); and *b*, the effective alveolar pressures are not derived from a spot sample expelled after a momentary delay but are derived from samples of arterial blood and expired air taken through several normal ventilatory cycles and are, therefore, representative of the mean physiological balance (time factor).

It would appear, then, that for investigations of the movement of O_2 and CO_2 and of the effect of alveolar gas pressures on arterial gas tensions (12), the effective (indirect) alveolar pressures are physiologically more significant than values obtained by presently available direct technics for sampling alveolar air.

SUMMARY

1. The limitations inherent in direct methods for sampling alveolar air become critical during experiments on exercising subjects.

2. An indirect method for calculating alveolar gas pressures has been devised which requires simply the determination of arterial $p\text{CO}_2$ and the $p\text{O}_2$ and $p\text{CO}_2$ of inspired and expired air.

3. A theoretical analysis indicates that alveolar gas pressures so determined represent the physiologically effective mean pressures and are not subject to errors introduced by "time" and "space" factors.

4. By this indirect method the effective alveolar pressures may be determined during exercise without encountering the difficulties inherent in the classical direct methods.

REFERENCES

- (1) BATEMAN, J. B. *Proc. Mayo Clin.* **20**: 214, 1945.
- (2) BERGGREN, S. M. *Acta Physiol. Scand.* **4**: Supplement 11, 1942.
- (3) BOCK, A. V., D. B. DILL, H. T. EDWARDS, L. J. HENDERSON AND J. H. TALBOTT. *J. Physiol.* **68**: 277, 1929.
- (4) BOOTHBY, W. M. *Proc. Mayo Clin.* **20**: 209, 1945.
- (5) COMROE, J. H., JR., AND R. D. DRIPPS, JR. *This Journal* **142**: 700, 1944.
- (6) FERGUSON, J. K. W. AND L. P. DUGAL. *Canad. J. Research, E.* **23**: 32, 1945.
- (7) HALDANE, J. S. AND J. G. PRIESTLEY. *Respiration*. Yale University Press, New Haven, 1935.
- (8) KROGH, A. AND J. LINDHARD. *J. Physiol.* **47**: 30, 1913.
- (9) KROGH, A. AND J. LINDHARD. *J. Physiol.* **47**: 431, 1914.
- (10) KROGH, A. AND J. LINDHARD. *J. Physiol.* **51**: 59, 1917.
- (11) KROGH, A. *The anatomy and physiology of the capillaries*. Yale University Press, New Haven, 1929. (*J. Physiol.* **52**: 391, 1919)
- (12) LILIENTHAL, J. L., JR., R. L. RILEY, D. D. PROEMMEL AND R. E. FRANKE. *This Journal* **147**: 199, 1946.
- (13) RILEY, R. L., D. D. PROEMMEL AND R. E. FRANKE. *J. Biol. Chem.* **161**: 621, 1945.
- (14) ROELSEN, E. *Acta Med. Scand.* **98**: 141, 1939.
- (15) SONNE, C. *Ztschr. ges. exper. Med.* **94**: 13, 1934.

AN EXPERIMENTAL ANALYSIS IN MAN OF THE OXYGEN PRESSURE GRADIENT FROM ALVEOLAR AIR TO ARTERIAL BLOOD DURING REST AND EXERCISE AT SEA LEVEL AND AT ALTITUDE¹

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The observation has been made that when man exercises during anoxia his cyanosis deepens (4, 11). This has been shown to reflect a corresponding fall in the oxyhemoglobin saturation of the arterial blood (16). In a recent study of this phenomenon it was reported that exercise during anoxia resulted in a fall in alveolar oxygen pressure but that the accompanying fall in oxygen saturation of the arterial blood "is greater than can be explained on the basis of the fall in oxygen tension of the alveolar air" (12). A possible explanation of this observation is the development during work at altitude of an appreciable oxygen pressure gradient between alveolar air and arterial blood.

The diffusion theory, which is accepted generally to explain the transfer of oxygen across the pulmonary "membrane", requires that the alveolar pO_2 exceed, by an indeterminate amount, the pO_2 of the blood leaving the alveolar capillary (5). This pressure gradient is not susceptible at present to direct experimental measurement in man. The size of the pressure gradient between alveolar air and *peripheral* arterial blood, on the other hand, has been the subject of several investigations, and widespread values from < 1 to > 60 mm. Hg have been recorded in normal man and animals at rest (2, 6, 7, 10, 13, 14). It has been difficult to reach any conclusion regarding the size of the alveolar-arterial (A-A) pO_2 gradient owing to uncertainties surrounding the estimation of a , the arterial gas tensions, and b , the alveolar gas pressures (19, p. 532).

Many of the studies of the A-A pO_2 gradient employed an indirect method to estimate the arterial pO_2 : the arterial oxyhemoglobin saturation was determined directly and the corresponding pO_2 was read off a standard dissociation curve. The uncertainties of this method for the high oxyhemoglobin range have been demonstrated recently (25). During anoxia with partial unsaturation of the arterial blood, however, the indirect estimation of pO_2 provides a high degree of accuracy, if the dissociation curve of oxyhemoglobin is not altered appreciably from its standard position at a given pH. But if a standard oxyhemoglobin dissociation curve were used when in fact the curve was shifted to the right, then the value of arterial pO_2 derived from the standard curve would be lower than the true value. This remotely possible source of error has been eliminated from consideration by a preliminary study which showed that oxyhemoglobin

¹ The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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dissociation characteristics remained constant during exercise and brief anoxia (20). The conclusion may be drawn, therefore, that an observed fall in arterial oxyhemoglobin saturation during exercise at altitude will reflect a true fall in arterial pO_2 . Whether or not a fall in arterial pO_2 may be taken to indicate a rise in the A-A pO_2 gradient depends on an accurate measurement of the accompanying changes in alveolar pO_2 .

Recent developments in this laboratory have made possible relatively precise measurements, during a variety of physiological stresses of *a*, the effective alveolar pressures of CO_2 and O_2 , and *b*, the arterial tensions of CO_2 and O_2 by direct means. This communication reports a study, by means of these technics, of the A-A pO_2 gradient in man at rest and during exercise, while breathing air at sea level and at simulated altitude.

METHODS. *Alveolar gas pressures.* The difficulties encountered in obtaining reliable samples of alveolar air at rest by the classical direct sampling methods increase when the subject exercises. In order to avoid these difficulties an indirect method for determining the effective alveolar gas pressures, which is described in the preceding paper, has been employed in the present study (21). The analytical error of the indirectly calculated effective alveolar pO_2 does not exceed 4 mm. Hg.

Arterial gas tensions. The blood sample was withdrawn from the brachial artery through an inlying needle which had been introduced earlier through an area of local anesthesia. The sample was analysed immediately for pCO_2 and pO_2 by the direct bubble method of Riley, Proemmel and Franke (22). The samples of blood were drawn at a steady rate during the minute period of collection of the expired air, and represented an average of the variations in gas tensions which might occur in response to the ventilatory cycle. The analytical error of the tension method does not exceed 3 mm. Hg.

Experimental design. The subjects were 6 healthy males whose vital measurements follow:

	Height (cm.)	Weight (kgm.)	Surface Area (sq.m.)	Age (yrs.)
C. H. F.....	188	69	1.92	28
C. S. H.....	174	71	1.84	32
J. L. L.....	178	78	1.96	34
R. L. R.....	174	62	1.74	34
T. J. R.....	173	69	1.81.	36
H. A. S.....	193	98	2.27	34

They were studied at rest while lying supine or standing quietly. Exercise was accomplished on a motor-driven treadmill moving at 4.8 km./hr. (3 m.p.h.), inclined upwards at 5 degrees (8.8 per cent grade). Exercise was continued before sampling until a "steady state" was reached (5 to 8 min.). The subjects breathed through a mouthpiece with nose-clip and were delivered either room air or oxygen-nitrogen mixtures exerting a tracheal³ pO_2 averaging 76 mm. Hg at rest and 90 mm. Hg at work. These pressures correspond approximately to

³37°C., saturated, ambient pressure.

pressure-altitudes of 5000 m. (16,500 ft.) and 4000 m. (13,000 ft.) respectively. Ventilation volumes were measured by delivering the inspired gases from a Tissot gasometer through appropriate valves, tubing, and reservoir bag which were so selected that there was no sensible resistance even when minute volumes exceeded 70 liters per minute. Expired gas was collected in a Douglas bag and the inspired and expired gases were analysed in a Haldane-Henderson analyser.

RESULTS. The results of 58 experiments are presented in table 1. There are certain relationships among these data which may be noted.

1. At rest, breathing air, the effective alveolar pO_2 averaged 103.5 mm. Hg (97–109) and the average arterial pO_2 was 94.2 (83–102), yielding an average total A-A pO_2 gradient of 9.3 mm. Hg. The mean of the individual averages was 8.9 mm. Hg.

2. An appreciable fall in the effective alveolar pCO_2 was observed during anoxia whether at rest (–5.3 mm. Hg) or at work (–6.8 mm. Hg).

3. There was a distinct but minor trend toward a reduction in oxygen consumption during anoxia as indicated by the average values, but the direction of change was not invariable.

4. The wide variation in individual responses to the stresses of work and anoxia is well demonstrated by comparing the data obtained from two subjects, J. L. L. and R. L. R. (table 1). Both these subjects had had extensive subjective experience with anoxia and with apparatus for respiratory measurements so that their responses were not likely to be distorted by an unfamiliar experimental environment. Note, for example, the responses of J. L. L. to exercise and to combined exercise and anoxia: the rises in the volume of minute ventilation over the resting rate were of the order of 9 X and 13 X respectively. Compare the responses of R. L. R. who, under the same circumstances, increased his ventilation 5 X and 9 X, responses which were only a little more than half as vigorous. Furthermore, comparison of experiments 30 and 31 with 32, 33 and 34 indicates another striking difference in these two subjects: J. L. L., while absorbing about 2 liters per minute of oxygen, developed a ventilation rate of about 45 liters per minute and an effective alveolar pO_2 of 110–112, but the arterial pO_2 did not rise above 81 mm. Hg. This subject developed an A-A gradient of 29–34 mm. Hg in meeting his metabolic requirements for oxygen. R. L. R., on the other hand, required 1.4 liters per minute of oxygen, ventilated 27 liters per minute, developed an alveolar pO_2 of 104–107 and maintained an arterial pO_2 of 91–93. The transfer of an adequate volume of oxygen required an A-A gradient of only 13 mm. Hg. Differences of this same order are apparent in the data recorded when these same subjects exercised during anoxia (expts. 47–52).

5. It must be emphasized that the values for the A-A gradients during exercise determined in these studies will hold only for oxygen consumption rates in the same general range. Examples of the change in gradient in response to a new work level are furnished in experiments 35, 36, 37, 53 and 54.

The range of the individual responses to a standard stress with respect to ventilation, maintenance of alveolar and arterial pO_2 levels and the A-A gradient

TABLE 1
Experimental data from 58 determinations of total A-A pO_2 gradient

SUBJECT	TRACHEAL pO ₂	ALVEO- LAR pCO ₂	ALVEO- LAR pO ₂	ARTER- IAL pO ₂	TOTAL A-A GRA- DIENT	RESPIRA- TORY QUOTIENT	OXYGEN CONSUMP- TION	VENTILA- TION RATE (STPD)
	In millimeters of mercury					In liters/min.		
	Rest—Air							
1. C. H. F.	151	36	108	102	6	0.845	0.300	6.5
2. C. S. H.	153	38	108	101	7	0.814	0.256	5.2
3. C. S. H.	153	37	106	98	8	0.788	0.237	4.9
4. C. S. H.	151	38	102	92	10	0.773	0.244	4.6
5. J. L. L.	152	41	98	86	12	0.757	0.282	4.8
6. J. L. L.	150	41	97	83	14	0.773	0.293	5.3
7. R. L. R.	152	36	106	95	11	0.786	0.242	5.3
8. R. L. R.	154	41	104	93	11	0.814	0.231	4.3
9. R. L. R.	154	36	109	95	14	0.805	0.253	4.8
10. T. J. R.	154	38	101	95	6	0.720	0.259	5.2
11. T. J. R.	154	43	102	95	7	0.749	0.253	5.2
12. H. A. S.	153	34	104	97	7	0.697	0.417	7.5
13. H. A. S.	153	33	101	93	8	0.634	0.353	5.8
Rest—Anoxia								
14. C. H. F.	78	35	45	40	5	1.058	0.324	11.0
15. C. S. H.	75	30	46	34	12	1.038	0.201	6.4
16. C. S. H.	75	30	53	45	8	1.383	0.180	8.3
17. C. S. H.	75	34	42	36	6	1.023	0.215	5.3
18. J. L. L.	77	32	47	36	11	1.060	0.289	8.8
19. J. L. L.	71	32	47	36	11	1.340	0.238	8.1
20. R. L. R.	77	36	40	31	9	0.980	0.194	5.9
21. R. L. R.	76	30	45	33	12	0.954	0.215	5.7
22. T. J. R.	80	32	49	39	10	1.044	0.177	4.9
23. T. J. R.	80	38	43	32	11	1.032	0.195	5.7
24. H. A. S.	77	34	43	34	9	1.002	0.359	10.0
25. H. A. S.	77	27	50	40	10	1.010	0.387	13.1
Exercise—Air								
26. C. H. F.	150	38	111	100	11	0.968	1.247	28.6
27. C. S. H.	153	45	99	89	10	0.828	1.242	20.0
28. C. S. H.	153	42	107	90	17	0.914	1.050	20.6
29. C. S. H.	153	40	103	90	13	0.810	1.242	22.5
30. J. L. L.	149	35	112	78	34	0.943	2.148	49.1
31. J. L. L.	149	37	110	81	29	0.949	1.995	42.5
32. R. L. R.	151	39	106	93	13	0.866	1.447	27.5
33. R. L. R.	151	39	107	94	13	0.882	1.425	27.3
34. R. L. R.	150	42	104	91	13	0.918	1.425	28.2
35. R. L. R.	*151	41	109	74	*35	0.986	2.840	55.9
36. R. L. R.	*151	39	110	72	*38	0.948	3.040	57.6
37. R. L. R.	*152	42	107	73	*34	0.927	2.960	56.8
38. T. J. R.	153	47	99	80	19	0.870	1.829	27.0
39. T. J. R.	154	46	100	84	16	0.850	1.670	25.0
40. H. A. S.	153	37	110	95	15	0.856	2.485	46.8
41. H. A. S.	153	42	102	91	11	0.801	2.145	35.7

TABLE 1—*Concluded*

SUBJECT	TRACHEAL pO ₂	ALVEO- LAR pCO ₂	ALVEO- LAR pO ₂	ARTER- IAL pO ₂	TOTAL A-A GRA- DIENT	RESPIRA- TORY QUOTIENT	OXYGEN CONSUMP- TION	VENTILA- TION RATE (STPD)
	In millimeters of mercury					In liters/min.		
	Exercise—Anoxia							
42. C. H. F.	91	33	58	49	9	0.995	1.205	31.3
43. C. H. F.	87	32	55	43	12	1.020	1.270	35.8
44. C. S. H.	90	37	50	38	12	0.922	1.272	27.6
45. C. S. H.	90	36	50	32	18	0.902	1.057	22.6
46. C. S. H.	92	36	52	35	17	0.905	1.276	27.0
47. J. L. L.	87	27	64	36	28	1.156	1.730	62.2
48. J. L. L.	87	30	62	33	29	1.222	1.725	66.1
49. J. L. L.	87	26	64	37	27	1.117	1.885	68.2
50. R. L. R.	92	34	58	42	16	1.007	1.365	33.6
51. R. L. R.	87	36	50	32	18	0.978	1.498	36.0
52. R. L. R.	90	37	52	33	19	0.970	1.407	33.0
53. R. L. R.	*111	39	74	47	*27	1.041	2.550	61.7
54. R. L. R.	*113	37	76	48	*26	1.003	2.453	56.0
55. T. J. R.	93	44	46	35	11	0.938	1.478	30.7
56. T. J. R.	91	44	48	34	14	1.013	1.365	30.8
57. H. A. S.	90	28	60	40	20	0.934	2.055	50.6
58. H. A. S.	90	28	60	42	18	0.942	2.070	52.1

* These 5 experiments were performed on an 11° (19 p.c.) grade at 5.6 km./hr. (3.5 m.p.h.) and the 5 data are not included in the averages listed below.

Summary

	REST		EXERCISE	
	Air	Anoxia	Air	Anoxia
Total A-A gradient				
C. H. F.....	6.0	5.0	11.0	10.5
C. S. H.....	8.5	8.5	13.5	15.5
J. L. L.....	13.0	11.0	31.5	28.0
R. L. R.....	12.0	10.5	13.0	18.0
T. J. R.....	6.5	10.5	17.5	12.5
H. A. S.....	7.5	9.5	13.0	19.0
Average.....	8.9	9.1	16.6	16.9
Alveolar pCO ₂ (mm. Hg)				
Average.....	37.8	32.5	40.7	33.9
Alveolar pO ₂ (mm. Hg)				
Average.....	103.5	45.8	105.4	55.3
Arterial pO ₂ (mm. Hg)				
Average.....	94.2	36.3	88.9	37.4
O ₂ consumption (l./min.)				
Average.....	0.285	0.258	1.665	1.521

emphasizes the multiplicity of factors which operate to serve the primary need, an adequate flow of oxygen from alveolar air to arterial blood. The interaction of these factors exemplifies Barcroft's apothegm, "Every adaptation is an integration" (3).

DISCUSSION. 1. *The origins of the total A-A pO_2 gradient.* The oxygen pressure gradient which exists between alveolar air and peripheral arterial blood is the resultant of several factors which fall into two main categories and are represented schematically in figure 1.

The first component of the total A-A gradient results from the sum of the resistances interposed by the tissue-fluid barrier which separates the alveolar

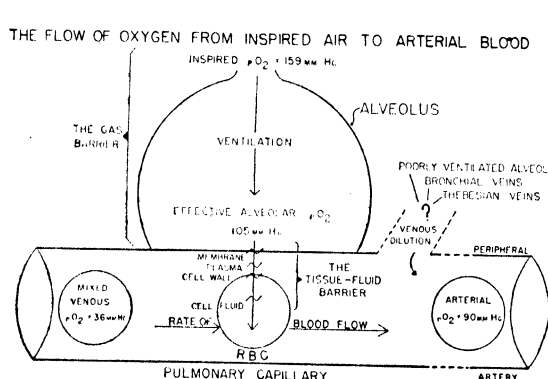


Fig. 1

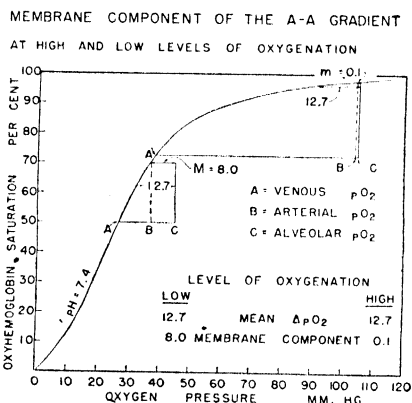


Fig. 2

Fig. 1. Some of the factors operating in the transfer of oxygen to the arterial blood.

Fig. 2. The effects of high and low level oxygenation on the membrane component of the total A-A pO_2 gradient. This graph illustrates the example calculated in the text (section 2B). At a constant work level the oxygen consumption is assumed to be the same at both levels of oxygenation and, therefore, the mean ΔpO_2 (12.7 mm. Hg) is the same at each level. It will be seen that these conditions are met only when the membrane component at low level oxygenation is large, $M = 8$ mm. Hg, while at high level oxygenation the membrane component must be very small, $m = 0.1$ mm. Hg.

At low level oxygenation venous $pO_2 = 27$, arterial $pO_2 = 37$ and alveolar $pO_2 = 45$ mm. Hg. At high level oxygenation venous $pO_2 = 38$, arterial $pO_2 = 104$ and alveolar $pO_2 = 104.1$ mm. Hg.

air from the hemoglobin molecule: capillary endothelium, plasma, erythrocyte wall and intracellular fluid. Because the behavior of this barrier may be described by the physicochemical laws pertaining to the diffusion of gases through membranes we will assign the term *pulmonary membrane* to the total tissue-fluid barrier, without implying that it is a discrete anatomical structure. No necessity arises to enter into the controversy regarding the existence of an alveolar epithelium; if such exists, the resistance offered by it would be included in the pulmonary membrane component, as would also the additional resistance created by intra-alveolar exudation or fibrosis. Furthermore, if the time required to reach oxygen equilibrium between plasma and intracellular hemoglobin

exceeds the time available within the alveolar capillary (7), then this factor likewise would contribute to the pulmonary membrane component.

The second component of the total A-A pO₂ gradient results from the addition of venous blood to arterialised blood. The sources of this venous admixture may be, among others, blood which has passed through poorly ventilated alveoli, blood entering downstream from the alveolar capillary from the bronchial veins (metabolism of the lung) and from anterior cardiac and Thebesian veins, or other possible anastomoses or shunts (9).

2. *The size of the components of the total A-A pO₂ gradient.* The experimental determination of the A-A pO₂ gradient yields only the sum of the membrane and the venous admixture components. But, if the A-A gradient is determined during both normal and anoxic conditions, it is possible then to differentiate the contributions to the total gradient of each of these main components. The basis of this differentiation is in consequence of the shape of the oxyhemoglobin dissociation curve which necessitates the following relationships: if the arterial pO₂ falls, the final oxygen pressure gradient across the tissue-fluid barrier (the membrane component) rises (2; pp. 71 and 175), while the contribution to the total A-A pO₂ gradient owing to the addition of incompletely arterialised blood (the venous admixture component) decreases (10). These relationships make it possible to evaluate the magnitude of each component (membrane and venous admixture) by estimating it at high and low levels of oxygenation.

In order to analyse the reciprocal changes which occur in the sizes of the membrane and venous admixture components when the level of oxygenation is altered certain general assumptions will be made:

i, that the total A-A pO₂ gradient is composed only of membrane and venous admixture components;

ii, that the oxyhemoglobin dissociation curves are accurate (20);

iii, that, for a given work level, the effective diffusing area and the resistance of the pulmonary membrane (see section 4 on the diffusion constant of the lung) remain constant at high and low levels of oxygenation;

iv, that, for a given work level, the proportion of blood flowing through poorly ventilated alveoli, through bronchial, anterior cardiac and Thebesian veins, and through anastomoses or shunts remains constant at high and low levels of oxygenation; and

v, that the total venous admixture component although arising from sources with various oxygen saturations may, nevertheless, be expressed as though it were all the result of the addition of mixed venous blood.

In addition, certain specific assumptions, based in part on the average values found in table 1 will be made:

1, that at rest, at the high level of oxygenation the arterial pO₂ is 95 mm. Hg and the relative arterio-venous (A-V) oxygen difference is 25 per cent (in terms of oxyhemoglobin saturation); and that at the low level of oxygenation the arterial pO₂ is 37 mm. Hg, and owing to the increased cardiac output induced by anoxia the A-V oxygen difference is 20 per cent;

2, that during exercise, at the high level of oxygenation, the arterial pO₂ is 89

mm. Hg and the A-V oxygen difference is 50 per cent; and that at the low level of oxygenation the arterial pO_2 is again 37 mm. Hg and the A-V oxygen difference is 40 per cent,

3, that the pHs of both arterial and venous blood remains at 7.4⁴, and,

4, that at high level oxygenation the pO_2 of the blood leaving the alveolar capillary is 1.0 mm. Hg less than the average effective alveolar pO_2 ; i.e., $105 - 1 = 104$ mm. Hg. (As will be seen below, the final estimate for the membrane component at high level oxygenation is less than 1 mm. Hg.)

A. *The venous admixture component.* Let us define the magnitude of the venous admixture component of the total A-A pO_2 gradient at high and low levels of oxygenation. If the blood which leaves the alveolar capillary has an oxygen tension of 104 mm. Hg then its oxyhemoglobin saturation, as read from a standard dissociation curve⁵, is 97.5 per cent. If the A-V oxygen difference is 25 per cent in terms of oxyhemoglobin saturation, then the mixed venous blood is 72.5 per cent saturated. If the blood in the peripheral artery is composed of 96.2 parts of arterialised blood and 3.8 parts of the mixed venous blood, the final mixture will have a saturation of $(0.962 \times 97.5) + (0.038 \times 72.5) = 96.6$ per cent, and will exert a pO_2 of 96 mm. Hg. Since the blood leaves the alveolus with a pO_2 of 104 mm., a 3.8 per cent venous inflow will create an admixture component of $104 - 96 = 8$ mm. Hg. When, under conditions of anoxia, the blood leaves the alveolus with a pO_2 of 37 mm. Hg and a saturation of 70 per cent, the A-V oxygen difference of 20 per cent fixes the composition of the mixed venous blood at a pO_2 of 27 mm. Hg and a 50 per cent oxyhemoglobin saturation. By assuming again that the peripheral arterial blood is diluted by a 3.8 per cent contribution of mixed venous blood and performing a calculation similar to that outlined above, we find that the oxygen tension of the mixed arterial blood will not fall 8.0 mm. Hg as before but only 0.7 mm. Hg.

B. *The pulmonary membrane component.* The experimental measurements in table 1 show that the total A-A gradient is not appreciably different at high and low levels of oxygenation. Since, under the conditions of the analysis above, the venous admixture component decreases from 8.0 to 0.7 mm. Hg and the total gradient remains essentially constant, the membrane component must increase at the lower level of oxygenation. Specifically, if the total A-A gradient is 9 mm. Hg at both levels of oxygenation (table 1), then the membrane component would appear to be $9.0 - 8.0 = 1.0$ mm. Hg at the upper level of oxygenation and $9.0 - 0.7 = 8.3$ mm. Hg at the lower level of oxygenation. While the size of the membrane component at the lower level is at first surprising, the theoretical necessity for such an increase during anoxia was suggested by Barcroft some years ago (2, pp. 71 and 175).

Let us analyse the problem by raising the following question: if the membrane

⁴Variations as large as 0.05 pH unit will produce no significant effect upon the values which are to be derived.

⁵The oxyhemoglobin dissociation curves employed in this study are based on the data obtained by D. B. Dill and are presented in the *Handbook of Respiratory Data in Aviation*, a restricted publication of the National Research Council.

component is assumed to be 8 mm. Hg during anoxia, what value would the membrane component be expected to have when the subject breathes air at sea level?

To answer this question it will be necessary to consider certain characteristics of the flow of oxygen from the alveolus into the blood. According to the diffusion theory, the rate at which oxygen flows across the pulmonary membrane is directly proportional to the difference in oxygen pressures on the two sides of the tissue-fluid barrier, the ΔpO_2 (15). As a unit of blood flows through the alveolar capillary and its pO_2 approaches that within the alveolus, the ΔpO_2 diminishes. But there is a theoretical *mean* ΔpO_2 such that, if it remained unchanged along the entire course of the capillary, oxygen would flow across the tissue-fluid barrier at its normal rate, and thus the total oxygen transfer per minute would remain constant. This theoretical mean ΔpO_2 can be estimated by Bohr's graphic integration method in which a key value is the final ΔpO_2 existing between alveolar air and the arterialised blood as it leaves the alveolar capillary (8). This final ΔpO_2 , existing at the distal end of the capillary, is the membrane component of the total A-A pO_2 gradient; and we are concerned here with the effect of changes in the level of oxygenation on this final ΔpO_2 .

Given a value for the membrane component at low level oxygenation, such as the 8 mm. Hg assumed, we can calculate that the corresponding mean ΔpO_2 is 12.7 mm. Hg. Since increasing the level of oxygenation does not alter the oxygen consumption greatly (table 1), and since by the diffusion theory the amount of oxygen crossing the tissue-fluid barrier is proportional to the mean ΔpO_2 , (provided that general assumption *iv* is valid) this value of mean ΔpO_2 can be used as a common factor in calculations at both levels of oxygenation. This relationship makes possible the estimation of that membrane component at high level oxygenation which corresponds to an 8 mm. Hg membrane component at low level oxygenation, in answer to the question posed above. The estimation is made by calculating for high level oxygenation the mean ΔpO_2 's which obtain for a series of possible membrane components. Then, by trial and error, that value for high level membrane component is found which determines a mean ΔpO_2 equal to the mean ΔpO_2 (12.7 mm. Hg) obtaining at low level oxygenation. The solution is unique: the membrane component to be expected at high level oxygenation is 0.1 mm. Hg, in contrast to the 8.0 mm. Hg gradient at low level oxygenation (fig. 2). It may be noted here that a membrane gradient of 0.1 mm. Hg is of the same order of magnitude but smaller than the values which both Marie Krogh and Barcroft concluded would describe best the conditions existing at sea level (2, 15).

We have seen that changes in oxygen tension produce opposite effects on the two main components of the total A-A pO_2 gradient: during anoxia the membrane component increases and the venous admixture component diminishes. Since the sum of these two components must equal the total A-A gradient as determined experimentally, it seems likely that the values which were assumed earlier in this discussion correspond closely to the existing orders of magnitude. At each of the levels of oxygenation which we have chosen, 95 and 37 mm. Hg, one

of the main components of the total A-A pO_2 gradient dwindles to insignificance while the other constitutes the major fraction of the total oxygen pressure gradient between alveolar air and arterial blood.

Since the general conclusions regarding the direction of change in the gradient components produced by changing levels of oxygenation do not depend upon the specific values of the assumptions made, they are valid descriptions of pressure relationships existing within a single or a group of identical alveolar-capillary units. Consideration of the effects of inequalities in ventilation-perfusion relationships and in membrane resistance indicates that the general conclusions

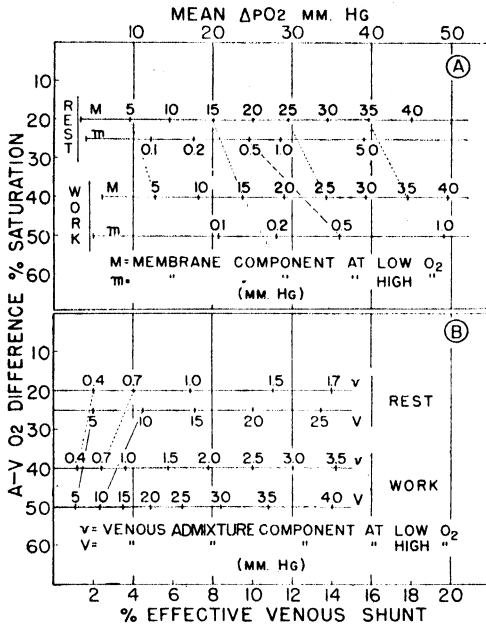


Fig. 3

Fig. 3. A. The relationship of the membrane component of the total A-A pO_2 gradient to mean ΔpO_2 . B. The relationship of the venous admixture component to the effective venous shunt.

Fig. 4. The oxygen tensions which obtain from alveolar air to peripheral arterial blood. X = exercise; ● = rest.

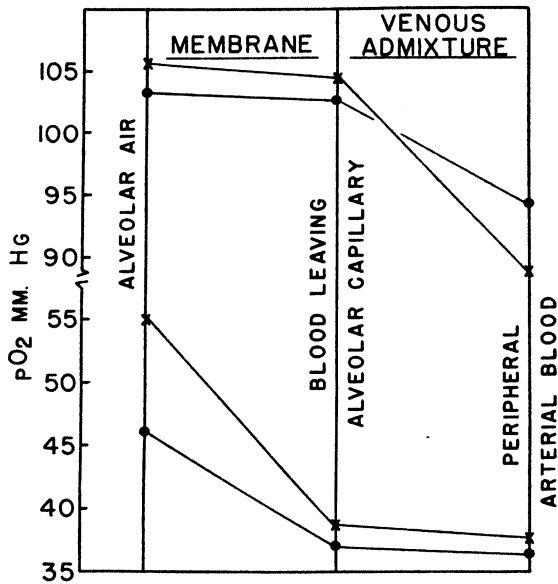


Fig. 4

remain valid but that the differentiation of membrane and venous admixture components by the use of high and low levels of oxygenation becomes slightly less sharp.

3. *Detailed procedure for differentiating experimentally determined A-A gradients.* Since the mean ΔpO_2 can be calculated for any given value of the membrane component, we may perform a series of such calculations using the basic assumptions listed in section 2 and determine the mean ΔpO_2 's which obtain for a variety of membrane component values at both high and low levels of oxygenation. The results may be plotted as in figure 3A. The A-V difference in percent satur-

ation, which appears as the ordinate, enters into the integration procedure by which the mean ΔpO_2 , the abscissa, is calculated. By plotting a wide range of calculations in this manner, the values of the membrane component at high level oxygenation which correspond to any given values at low level oxygenation can be read off directly along the same abscissa (mean ΔpO_2). The necessity for trial and error calculations thus is eliminated.

In section 2A, above, a method was described for calculating the change in the pO_2 of the arterialised blood which results from an admixture of venous blood. Let us then perform a series of calculations to determine what is the effective venous shunt for each of a number of venous admixture components at both high and low levels of oxygenation. When these calculations are charted as in figure 3B, the percentages of effective venous shunt appear along the abscissa, and, as in figure 3A, the A-V differences in per cent saturation are plotted along the ordinate.

Figure 3 now can be used to differentiate the experimentally determined A-A gradient into its two main components, membrane and venous admixture. If M and v represent the membrane and the venous admixture components at low level oxygenation, and m and V represent the respective components during high level oxygenation, then $M + v$ = total A-A gradient (low O_2) and $m + V$ = total A-A gradient (high O_2). Since M = total A-A gradient $-v$, and v is very small, M is approximately equal to the total A-A gradient at low level oxygenation. This approximate value for M can be used to determine m without significant error by reading off the value of m along the same abscissa (mean ΔpO_2) (fig. 3A). For example, when M at rest = 9.0 mm. Hg, m = 0.1. Now, since V = total A-A gradient $-m$, V can be estimated by direct substitution in the equation; v can then be read off along the same abscissa (percent effective venous shunt) in figure 3B.⁶ For example, V = 9.0 $-$ 0.1 = 8.9 and v = 0.7. Finally, M can be estimated with greater accuracy by substitution in the equation M = total A-A gradient $-v$. The final differentiation may be summarised as follows: for low oxygenation M = 8.3, v = 0.7, total A-A gradient = 9.0; and for high oxygenation m = 0.1, V = 8.9, total A-A gradient = 9.0.

In table 2 the average A-A gradients determined for the 6 subjects are differentiated by the method just described. Columns 2 through 7 are arranged in the order in which the corresponding steps have been taken in the procedure. The average values approximate closely those which are used in the example given above and illustrate the manner in which the two levels of oxygenation may be employed to reduce first one component and then the other to insignificance.

⁶ When m and v are read off along the same abscissae as M and V , the assumptions are made that, for successive experiments at a given work level, the mean ΔpO_2 and the effective venous shunt remain relatively constant. Because of the very small values of m and v , it is apparent that no significant errors will be introduced even if the assumptions in a specific instance should not be strictly accurate. It is also apparent that, if the oxyhemoglobin saturations of both arterial and mixed venous blood should be known (as in the direct Fick procedure) then the determined A-V difference in per cent saturation would be used in place of our assumed values.

A graphic summary of the gradient components is set forth in figure 4, where the values are calculated from the data available in tables 1 and 2. The alveolar pO_2 and the peripheral arterial pO_2 are the averages found in the summary of table 1; the average pO_2 of the blood leaving the alveolar capillaries is equivalent to the average alveolar pO_2 less the average value of m or M , depending upon the level of oxygenation. At high level oxygenation it will be noted that almost all of the fall in oxygen tension occurs after the blood has left the alveolar capillary

TABLE 2
A-A pO_2 gradient components and diffusion constants

1	2	3	4	5	6	7	8	9	10
Subject	A-A gradient (low O_2) M (approx.)	m (from fig. 5A)	A-A gradient (high O_2)	V (4-3)	g (from fig. 5B)	M (2-6)	Mean ΔpO_2 (from fig. 5A)	Oxygen consumption during anoxia (l./min)	Diffusion constant (Do ₂) ($9 + 8$) $\times 10^3$
Rest									
C. H. F.....	5	0.1	6	5.9	0.5	4.5	9	0.324	36
C. S. H.....	9	0.1	8	7.9	0.6	8.4	13	0.199	15
J. L. L.....	11	0.2	13	12.8	0.9	10.1	14.5	0.269	19
R. L. R.....	11	0.2	12	11.8	0.8	10.2	14.5	0.205	14
T. J. R.....	11	0.2	7	6.8	0.5	10.5	15.0	0.186	12
H. A. S.....	10	0.1	8	7.9	0.6	9.4	14.0	0.373	27
Average.....	9.5	0.2	9	8.8	0.7	8.9	13.3	0.258	21
Exercise									
C. H. F.....	11	0.1	11	10.9	0.8	10.2	18.5	1.238	67
C. S. H.....	16	0.2	13	12.8	0.9	15.1	24.0	1.202	50
J. L. L.....	28	0.6	32	31.4	2.1	25.9	35.0	1.780	51
R. L. R.....	18	0.2	13	12.8	0.9	17.1	26.0	1.423	55
T. J. R.....	13	0.1	18	17.9	1.2	11.8	20.0	1.422	71
H. A. S.....	19	0.2	13	12.8	0.9	18.1	27.0	2.063	76
Average.....	18	0.2	17	16.6	1.1	16.4	25.0	1.521	62
Strenuous exercise									
R. L. R.....	27	0.6	36	35.4	2.8	24.2	34.0	2.496	73

bed, while at low level oxygenation most of the total A-A gradient occurs across the pulmonary membrane. The effect of exercise on expanding the gradient is apparent.

It is of practical importance to note that the levels of oxygenation used in the analysis presented in this paper need not be reached exactly in order to apply the differentiation technic. The values used are, we believe, optimal because between these levels there is a maximal change in the slope of the oxyhemoglobin dissociation curve which is consistent with physiological oxygen requirements

and technical limitations (see fig. 2). However, because of the general finding that the total A-A gradient is not altered markedly by the change in level of oxygenation, it can probably be assumed that variations up to ± 10 mm. Hg from the levels of arterial pO₂ selected in this paper will not cause any significant alteration in the total A-A gradients from what they would have been at 95 and 37 mm. Hg respectively. The use of figure 3 to differentiate the A-A gradient will yield values for the membrane and venous admixture components which would have obtained at arterial pO₂'s of 95 and 37 mm. Hg and not those which actually obtained during the specific experiments. The use of values for membrane component so derived will not lead to significant error in the estimation of mean ΔpO_2 .

The experimental observations and theoretical assumptions used in differentiating the A-A pO₂ gradient may be summarised thus:

a. All factors which contribute to the A-A gradient fall into two categories which we have termed the membrane and venous admixture components respectively (section 1).

b. The experimentally determined total A-A pO₂ gradient averages about 9 mm. Hg at rest at both high and low levels of oxygenation (table 1).

c. Theoretical considerations suggest that, for a given work level, the mean ΔpO_2 remains approximately constant at high and low levels of oxygenation (section 2B).

d. When mean ΔpO_2 is kept constant, a membrane component of 8 mm. Hg during anoxia dwindles to less than 1 mm. Hg at high level oxygenation, as calculated by a graphic integration procedure (section 2B).

e. Theoretical considerations suggest that, for a given work level, the amount of venous admixture to the arterialised blood, expressed as an effective shunt of mixed venous blood, remains approximately constant at high and low levels of oxygenation (section 2A).

f. When the effective venous shunt is kept constant, a venous admixture component of 8 mm. Hg at high level oxygenation dwindles to less than 1 mm. Hg during anoxia, as calculated by simple mixing equations (section 2A).

g. These relationships force the conclusion that the total A-A gradient is virtually all membrane component at low level oxygenation, and that the venous admixture component constitutes virtually all of the total gradient at high level oxygenation (section 2).

h. By using mathematical calculations to estimate the smaller component at each level of oxygenation, a differentiation of the total A-A pO₂ gradient into membrane and venous admixture components can be made by starting from experimentally determined total gradients at high and low levels of oxygenation (section 3).

4. *The diffusion constant of the lung.* The diffusion constant of the lung (D_{O_2}) is defined as the number of cubic centimeters of oxygen crossing the pulmonary membrane per minute in response to a mean pressure gradient of 1 mm. Hg (15). Thus, $D_{O_2} = O_2 \text{ consumption per minute} \div \text{mean } \Delta pO_2$. Since in the process of differentiating the A-A gradient we have calculated mean ΔpO_2 , and since the

oxygen consumption per minute was measured, the necessary data are at hand for calculating D_{O_2} . These calculations are presented in table 2. Among our normal subjects, D_{O_2} at rest averaged 21 (12 to 36) and D_{O_2} during exercise averaged 62 (50 to 76).

The value of the diffusion constant in man and the adequacy of the diffusion theory in accounting for oxygen transfer during exercise are subjects which have evoked much interest and controversy. Although the basic theory was propounded clearly many years ago (15), the technical methods available were inadequate to establish by direct measurement the quantitative relationships in man. The methods for measuring alveolar pO_2 and arterial pO_2 which have been employed in the present studies make possible a more direct evaluation of the conditions determining the diffusion of oxygen in the lungs. Furthermore, experimental data are now at hand which make possible a differentiation of the total A-A gradient and a quantitative estimation of the membrane component. This membrane component is a critical factor in the calculation of mean ΔpO_2 by Bohr's method and hence in the determination of the diffusion constant.

Marie Krogh first calculated oxygen diffusion constants in man indirectly from measurements of the rate of carbon monoxide diffusion from the alveolar air, and found D_{O_2} to range from 23 to 43 at rest and 37 to 56 at work. Roughton, using Krogh's values for D_{O_2} , was unable to account for the high rates of oxygen transfer which obtain during work (23). In his calculations he made use of the data which Asmussen and Chiodi recorded in subjects working at a simulated altitude of 6000 m. (20,000 ft.) (1). These conditions corresponded closely to those of our experiments where the subjects exercised during anoxia (low level of oxygenation). In calculating the mean ΔpO_2 which existed during the experiments of Asmussen and Chiodi, Roughton appears to have assigned a value of approximately 2 mm. Hg to the pulmonary membrane gradient, in accord with the generally accepted estimate, and thus obtained a D_{O_2} of 200. This was 3 times greater than the highest D_{O_2} to be expected from Marie Krogh's measurements of the D_{CO} . The data obtained in our experiments suggest that in Asmussen and Chiodi's experiments the membrane component (M) amounted more probably to 16.4 mm. Hg (table 2), the mean ΔpO_2 to 25 mm. Hg (fig. 3A) and the D_{O_2} to 64 when the oxygen consumption was 1.6 l./min. (fig. 5). Our calculations based on the oxygen relationships, are in full agreement with those which Marie Krogh deduced so brilliantly from measurements of the diffusion constant for carbon monoxide (D_{CO}).

The relatively simple process of diffusion of oxygen across the pulmonary membrane to combine with hemoglobin is subserved and limited by a series of inter-related functions. There are important factors of intra-pulmonary ventilation and gas mixing which determine, in part, the alveolar environment (the gas barrier in fig. 1), but we are concerned here only with a description of the events occurring after the constitution of the alveolar air. A most instructive method for visualising the inter-relations of the functions concerned in the delivery of oxygen was designed by Murray and Morgan (18) and later amplified by Barcroft (3; fig. 79). We have modified this method in figure 5 to demonstrate

the integration of the membrane component of the total A-A gradient with related functions: oxygen consumption, cardiac output, absolute A-V oxygen difference (vols. per cent), oxyhemoglobin capacity, relative A-V oxygen difference (per cent saturation), mean ΔpO_2 and the diffusion constant.

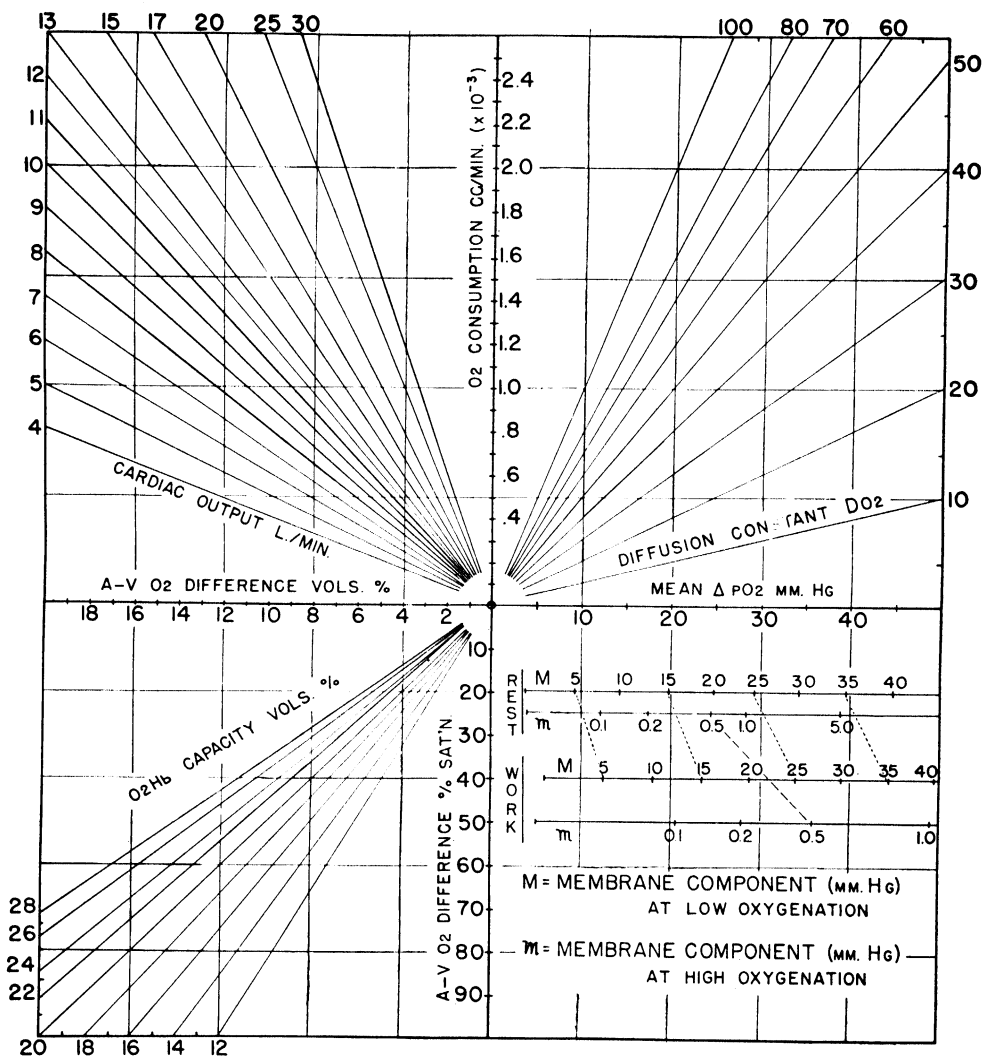


Fig. 5. The integration of related respiratory and cardiovascular functions and their adaptation to stress. Modified after Murray and Morgan (18) and Barcroft (3).

The functions described in figure 5 must always maintain a quadrangular relationship; and the adjustments to stress result in an expansion of the quadrangle. Thus, the increased demand for oxygen which accompanies exercise is answered by a rise in cardiac output, a larger volume A-V oxygen difference, a

slight increase in oxyhemoglobin capacity, with a corresponding increase in the A-V oxygen saturation difference. These changes are associated with an increase in both the membrane component of the total A-A gradient and the mean ΔpO_2 and this in turn requires an appreciable increase in the size of the diffusion constant.

It may be noted from table 2 and figure 5 that one of the adjustments to a more urgent demand for oxygen is a two- to threefold increase in the diffusion constant of the lung, the D_{O_2} . The means by which this expansion of the diffusing capacity might be effected are of considerable interest. The diffusion constant of the lung may be considered in general terms to be the product of the permeability of the pulmonary membrane per unit area *times* the total diffusing area (the active capillary surface area). Changes in D_{O_2} then may result from changes in either or both of these two factors which determine it. It seems unlikely, on the basis of present evidence, that the permeability of the membrane per se increases in response to the heightened demand for oxygen. A more probable adaptation is an increase in the area of the diffusing bed effected by an exposure of a larger volume of blood to alveolar air. An enlargement of the volume of blood so exposed might result from two mechanisms: an accelerated flow of blood through the alveolar bed or by an expansion of the active alveolar bed. This latter mechanism could be the result of a) an opening of additional complete alveolar units, b) an opening of additional capillaries in existing alveoli, or c) an increase in the cross-section area of the existing capillary bed.

The experimental evidence regarding the adjustments of the pulmonary circulation to various stresses is conflicting (17; Ch. 5), but there is reason to believe that the rate of blood flow through the alveolar bed must rise. Furthermore, Roughton has adduced indirect evidence recently which indicates that, when compared to the values obtaining at rest, exercise of the order which we have studied here produces these effects: *a*, the time spent by the blood in the alveolar bed is halved, and *b*, the volume of blood in patent alveolar capillaries is increased some 60 per cent (24). The magnitude of these changes during exercise will account for a large part of the increase in D_{O_2} which in our experiments amounts to some 200 per cent.

In all likelihood the integrated adaptation to stress, indicated by the rise of the diffusion constant, makes use of all these available mechanisms and perhaps others, to varying degrees depending upon the individual and the severity of the stress.

SUMMARY

1. By means of new technics, measurements have been made in man of the oxygen pressure gradient existing between the alveolar air and the peripheral arterial blood, during rest and exercise, at sea level and at simulated altitude.
2. At rest the gradient averaged 9 mm. Hg and during exercise 16.5 mm. Hg; the development of anoxia produced no significant changes in the size of the gradients.
3. A method is presented for differentiating the total alveolar arterial oxygen

pressure gradient into its two main components: membrane resistance and venous admixture.

4. A theoretical analysis of the experimental data indicates that when the level of oxygenation was high (sea level) the observed pressure gradient resulted for the most part from the admixture of venous blood entering from poorly ventilated alveoli, the bronchial circulation, the Thebesian and anterior cardiac veins and perhaps other sources. By contrast, at low level oxygenation (anoxic anoxia) the gradient resulted largely from the pressure head which must develop across the pulmonary membrane to effect the transfer of the required volume of oxygen.

5. Exercise, by exerting a physiological stress on the mechanisms serving the transfer of oxygen from alveolar air to arterial blood, evokes an integrated series of respiratory and cardiovascular adaptations, one of which is the increase of the alveolar-arterial oxygen pressure gradient.

6. The diffusion constant of the lung, calculated from the experimental observations, averaged 21 at rest (range 12 to 36) and increased during exercise to an average of 62 (range 50 to 76).

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REFERENCES

- (1) ASMUSSEN, E. AND H. CHIODI. *This Journal* **132**: 426, 1941.
- (2) BARCROFT, J. *The respiratory function of the blood. I. Lessons from high altitudes.* University Press, Cambridge, 1925.
- (3) BARCROFT, J. *Features in the architecture of physiological function.* University Press, Cambridge, 1938.
- (4) BARCROFT, J., C. A. BINGER, A. V. BOCK, J. H. DOGGART, H. S. FORBES, G. HARROP, J. C. MEAKINS AND A. C. REDFIELD. *Phil. Trans. Roy. Soc. (London) B.* **211**: 351, 1923.
- (5) BARCROFT, J., A. COOKE, H. HARTRIDGE, T. R. PARSONS AND W. PARSONS. *J. Physiol.* **53**: 450, 1920.
- (6) BERGGREN, S. M. *Acta Physiol. Scand.* **4**: Supplement 11, 1942.
- (7) BOCK, A. V., D. B. DILL, H. T. EDWARDS, L. J. HENDERSON AND J. H. TALBOTT. *J. Physiol.* **68**: 277, 1929.
- (8) BOHR, C. *Skand. Arch. f. Physiol.* **22**: 221, 1909.
- (9) DALY, I. DEB. *Harvey Lect.* **31**: 235, 1936.
- (10) DILL, D. B., E. H. CHRISTENSEN AND H. T. EDWARDS. *This Journal* **115**: 530, 1936.
- (11) DOUGLAS, C. G., J. S. HALDANE, Y. HENDERSON AND E. C. SCHNEIDER. *Phil. Trans. Roy. Soc. (London) B.* **203**: 185, 1913.
- (12) GEMMILL, C. L. *Fed. Proc.* **4**: 23, 1945.
- (13) GREENE, C. W. AND C. H. GREENE. *J. Biol. Chem.* **52**: 137, 1922.
- (14) KROGH, A. AND M. KROGH. *Skand. Arch. f. Physiol.* **23**: 179, 1910.
- (15) KROGH, M. *J. Physiol.* **49**: 271, 1915.

- (16) LILIENTHAL, J. L., JR., R. L. RILEY AND D. D. PROEMMEL. This Journal **145**: 427, 1946.
- (17) McDOWALL, R. J. S. The control of the circulation of the blood. Longmans, Green & Co., London, 1938.
- (18) MURRAY, C. D. AND W. O. P. MORGAN. J. Biol. Chem. **65**: 419, 1925.
- (19) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. I. Interpretations. Williams and Wilkins Co., Baltimore, 1932.
- (20) RILEY, R. L., J. L. LILIENTHAL, JR., D. D. PROEMMEL AND R. E. FRANKE. J. Clin. Investigation **25**: 139, 1946.
- (21) RILEY, R. L., J. L. LILIENTHAL, JR., D. D. PROEMMEL AND R. E. FRANKE. This Journal **147**: 191, 1946.
- (22) RILEY, R. L., D. D. PROEMMEL AND R. E. FRANKE. J. Biol. Chem. **161**: 621, 1945.
- (23) ROUGHTON, F. J. W. Am. J. Med. Sci. **208**: 136, 1944.
- (24) ROUGHTON, F. J. W. This Journal **143**: 621, 1945.
- (25) ROUGHTON, F. J. W., R. C. DARLING AND W. S. ROOT. This Journal **142**: 708, 1944.

CARBON DIOXIDE PRODUCTION OF HUMANS AT SEA LEVEL AND AT LOW BAROMETRIC PRESSURES¹

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The resting metabolism of humans, as measured by oxygen consumption is reported to be decreased after decompression to low barometric pressures (Cook, 1945). This effect is attributed to a specific action of the low pressure on metabolism; a theory which is supported by experiments on animals and tissues (Cook, 1931). That the effect is in controversy is evidenced by the reports of Eckman et al. (1945) and Nims (1945) who were unable to demonstrate a decrease in basal oxygen consumption at altitude. The results of Cook were criticized because the subjects were not under basal conditions, and in addition there may have been errors resulting from temperature fluctuations in the metabolism apparatus.

The present experiments were undertaken to utilize measurements of resting carbon dioxide production of humans under basal and non-basal conditions, as indicative of the metabolism at sea level and altitude. The methods used for measuring carbon dioxide production are more direct compared to measuring oxygen consumption, and for this reason errors (such as temperature and water vapor effects) in measuring metabolism by oxygen consumption methods are avoided.

METHODS. The arrangement for collecting the expired gases in Douglas bags at sea level and altitude is illustrated in figure 1.

The following procedure was used for experiments with subjects under either basal or non-basal conditions. The subject was allowed to rest lying down for 20-30 minutes at sea level pressure. A metabolism mask was fitted to the face allowing pure oxygen to be breathed from an oxygen demand regulator. By means of a one way valve the expired gases were forced along on exhaust tubing to a three way valve to which could be attached a Douglas bag. The expired gases were collected over a period of 15-20 minutes at sea level. The percentage carbon dioxide in the expired gases was determined by a Haldane gas analyzer, and the volume measured in a large spirometer. At the termination of the sea level measurement the subject was decompressed to a pressure equivalent to 30,000 feet and after a few minutes the expired gases collected in a Douglas bag for 18-25 minutes. Unless the subject experienced difficulties (such as ear or sinus trouble) during recompression another control measurement was made at sea level.

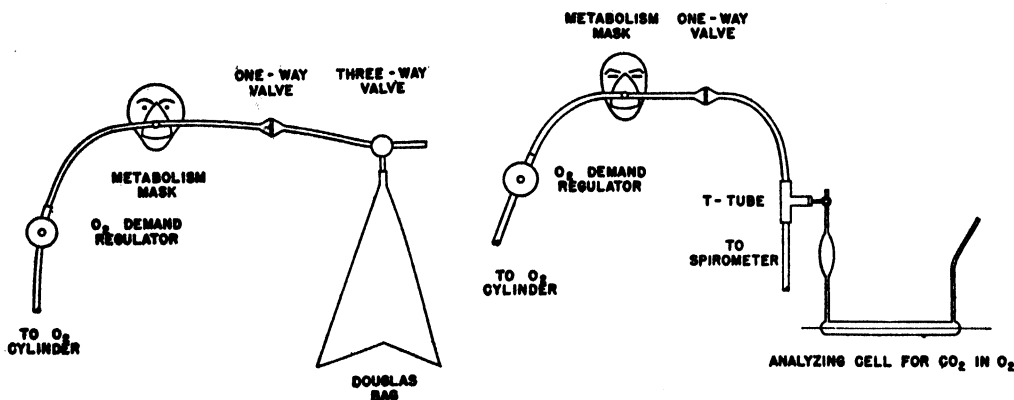
The total time for the altitude and two control measurements was approxi-

¹ The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of California.

mately one and one half hours. The subjects were recumbent and breathed pure oxygen during this entire period.

A second method for measuring carbon dioxide elimination employed a thermal conductivity gas analyzer for carbon dioxide in oxygen (Berg, 1945). This arrangement as shown in figure 2 involved continuous measurement of the per cent carbon dioxide in the expired air and determination of the minute ventilation with a large spirometer. By this means the analyses and volume measurements could be made directly at altitude.

The experimental error when using the Douglas bags is low (< 1 per cent) since the collected gases are analyzed and volume measurements made at sea level under the same conditions. The carbon dioxide is determined to within ± 0.03 per cent and the volume of expired gas is measured with an accuracy of ± 0.05 liter.



The experimental error using the thermal conductivity analyzer at sea level and at altitude is more difficult to evaluate since fluctuations of temperature may alter minute ventilation readings. Corrections were made for this and the total error is probably less than ± 2 per cent.

RESULTS. Data obtained on the carbon dioxide elimination of subjects under nonbasal conditions are listed in tables 1-3. These experiments were carried out from 1:30 p.m. to 3:00 p.m. Data on subjects under basal conditions are listed in table 4. The two control values of carbon dioxide elimination as listed in these tables were averaged and the difference from the altitude value expressed as cubic centimeters $\text{CO}_2/\text{minute S.T.P.}$

The data listed in tables 2 and 3 are statistically significant in showing a decrease in carbon dioxide production at 30,000 feet. The data in table 1 are significant in showing a decrease in carbon dioxide production at 30,000 feet; however, they give no information concerning the magnitude of the decrease in metabolism due to the prolonged resting. The differences in the carbon dioxide production between the two control runs in tables 2 and 3 are not signifi-

cant (Critical Ratio = 1.43), which therefore indicates that the significant decrease as shown in table 1 is a change of carbon dioxide production due to decompression.

TABLE 1

This series includes all experiments with a single control measurement of carbon dioxide production at sea level. Included are experiments where the second sea level determination could not be made due to difficulty in recompression of the subject.

SUBJECT	CO ₂ /MIN. (S.T.P.) PRODUCED AT SEA LEVEL	CO ₂ /MIN. (S.T.P.) PRODUCED AT 30,000 FT.	DIFFERENCE, CO ₂ /MIN.
	cc.	cc.	
F. T.....	187	168	-19
D. L.....	277	274	-3
E. M.....	214	186	-28
E. S.....	193	173	-20
S. C.....	185	165	-20
E. M.....	189	158	-31
S. C.....	202	186	-16
E. M.....	192	165	-27
B. B.....	232	197	-35
S. C.....	200	183	-17
E. M.....	198	170	-28

C.R. of difference = 8.31 (significant at the 1 per cent level).

TABLE 2

Two control experiments were carried out—one before and one after the determination at altitude. The expired gases were collected in Douglas bags and the Haldane gas analyzer was used for determination of carbon dioxide.

SUBJECT	CO ₂ /MIN. (S.T.P.) PRODUCED AT SEA LEVEL	CO ₂ /MIN. (S.T.P.) PRODUCED AT 30,000 FT.	CO ₂ /MIN. (S.T.P.) PRODUCED AT SEA LEVEL	DIFFERENCE, CO ₂ /MIN.
	cc.	cc.	cc.	
S. C.....	195	182	191	-11
E. S.....	211	201	217	-13
E. M.....	196	182	185	-9
E. S.....	246	201	220	-32
H. J.....	246	213	249	-35
E. D.....	296	244	291	-49
D. L.....	245	217	244	-27
F. T.....	212	181	207	-28
C. T.....	207	221	242	±0
W. B.....		193	211	-18

C.R. of difference = 4.79 (significant at the 1 per cent level).

The average decrease of carbon dioxide production at 30,000 feet as measured by use of Douglas bags and the Haldane gas analyzer is 9.5 per cent (table 2); the average decrease using the thermal conductivity analyzer is 7.7 per cent (table 3). These two methods of measuring carbon dioxide production involve

different principles and are subject to different errors—yet the results show a close check.

The data of Eckman and of Nims were obtained on subjects under basal conditions, whereas those reported by Cook (and also the present results as listed in tables 1-3) were obtained on subjects under non-basal conditions.

TABLE 3

Two control experiments were performed—one before and one after the altitude measurement of carbon dioxide production. The thermal conductivity method of analysis was used, the analyses being made directly at altitude.

SUBJECT	CO ₂ /MIN. (S.T.P.) PRODUCED AT SEA LEVEL	CO ₂ /MIN. (S.T.P.) PRODUCED AT 30,000 FT.	CO ₂ /MIN. (S.T.P.) PRODUCED AT SEA LEVEL	DIFFERENCE, CO ₂ /MIN.
	cc.	cc.	cc.	cc.
E. M.....	200	182	187	-11
F. T.....	190	173	186	-15
E. M.....	223	198	202	-14
W. W.....	211	179	201	-27
B. B.....	223	231	237	±0
S. C.....	224	210	210	-7
E. S.....	275	215	232	-38
E. M.....		179	198	-19

C.R. of difference = 3.91 (significant at the 1 per cent level).

TABLE 4

The subjects were under basal conditions. Two control determinations of carbon dioxide production were made. The expired gases were collected in Douglas bags and the Haldane gas analyzer was used for determination of carbon dioxide.

SUBJECT	CO ₂ /MIN. (S.T.P.) PRODUCED AT SEA LEVEL	CO ₂ /MIN. (S.T.P.) PRODUCED AT 30,000 FT.	CO ₂ /MIN. (S.T.P.) PRODUCED AT SEA LEVEL	DIFFERENCE, CO/ MIN.
	cc.	cc.	cc.	cc.
E. M.....	160	154	157	-4
W. W.....	174	166	159	±0
E. D.....	222	200	223	-22
H. J.....	219	186	205	-26
S. C.....	148	146	155	-6
B. B.....	189	187	184	±0
W. S.....	182	156	166	-18
F. T.....	168	156	176	-16
H. S.....	234	205	216	-20

C.R. of difference = 3.60 (significant at the 1 per cent level)

Measurements of carbon dioxide production were therefore carried out on subjects under basal conditions. The results as listed in table 4 show that at 30,000 feet a significant decrease occurs in basal carbon dioxide production. The average decrease for nine subjects was 6.4 per cent which is comparable to the data obtained on subjects under non-basal conditions.

SUMMARY

The carbon dioxide elimination of humans, either under basal or non-basal conditions, is significantly decreased at a simulated altitude of 30,000 feet as compared with the sea level values.

Two methods for measuring the carbon dioxide elimination were used. One method involved collection of the expired gases in Douglas bags, the analyses being made at sea level with the Haldane gas analyzer. The second method employed a thermal conductivity analyzer for carbon dioxide in oxygen; the analyses and volume measurements were made directly at altitude.

The average decrease of carbon dioxide elimination of non-basal subjects at altitude as measured by these methods was 8.6 per cent. The average decrease for subjects under basal conditions was 6.4 per cent.

REFERENCES

- BERG, W. E. Aero Medical Unit, University of Calif., Berkeley, Calif. (unpublished), 1945.
- COOK, S. F. AND G. GIRAGOSSINTZ. University of Calif. Publ. Physiol. 7: 237, 1931.
- COOK, S. F. J. of Aviation Med. 16: 268, 1945.
- ECKMAN, M. Aviation Research Laboratory, Columbia University (unpublished), 1945.
- NIMS, L. F. AND F. OLMSTED. Aero Medical Unit, Yale University (unpublished), 1945.

THE EFFECT OF ADRENALECTOMY IN RATS ON URINARY NON-PROTEIN NITROGEN DURING FORCED-FEEDING AND DURING FASTING

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This study is one of a series planned to test the ability of the rat to make metabolic adjustments following adrenalectomy. Ingle (1) has reviewed the evidence that adrenalectomized animals are defective in their ability to utilize fed protein and to mobilize their own tissue proteins during fasting and other forms of stress. In the present experiments adrenalectomized animals excreted as much urinary non-protein nitrogen as their controls during the post-operative period of forced-feeding and during the first several days of fasting.

METHODS. Male rats of the Sprague-Dawley strain which were completely free from infections were used. The stock diet was Purina Dog Chow. When the rats reached a weight of approximately 300 grams they were adapted to the forced-feeding of either a medium carbohydrate or a medium protein diet administered by stomach tube each morning (8:30 to 9:15 a.m.) and afternoon (4:15 to 5:00 p.m.) The technique of forced-feeding and the diets used (table 1) were modifications of those described by Reinecke, Ball and Samuels (2). The rats were brought to a full feeding on the fifth day. After the rats had been force-fed for two weeks the adrenal glands were removed by the procedure described by Ingle and Griffith (3). In the control animals the adrenal glands were exposed but were not damaged. Asepsis was successfully maintained in these operations. All of the animals were given a solution of 1 per cent sodium chloride to drink during all phases of the experiments. The animals were housed in an air-conditioned room in which the temperature was maintained at 74 to 78 degrees F. and the humidity at 30 to 35 per cent of saturation.

Twenty-four hour samples of urine were collected at the same hour each day and preserved with thymol and added citric acid (1 gram per sample) to insure the acidity of the urines for nitrogen analysis. The Miller and Van Slyke method (4) was used for the determination of blood glucose. The determination of urinary non-protein nitrogen was by the micro-Kjeldahl procedure as follows: proteins were precipitated as the salts of tungstic acid by the Folin-Wu procedure. The organic matter was oxidized by sulfuric acid and hydrogen peroxide. The ammonia was distilled off into a standard acid solution and titrated with standard base.

EXPERIMENTS AND RESULTS. In experiment 1 (fig. 1), 15 rats were adapted to the forced-feeding of the medium carbohydrate diet for 2 weeks. Eight rats were then adrenalectomized and 7 rats were sham-operated. Forced-feeding was continued for 2 weeks post-operatively and all of the animals were then fasted for 10 days. All of the animals received saline to drink during each

phase of the experiment. During the first post-operative day the adrenalectomized rats excreted less nitrogen; but by the third day there was a rise in urinary nitrogen which was sustained for several days and was greater in the adrenalectomized rats than in the controls. During the 2 weeks of post-operative feeding the urinary nitrogen of the adrenalectomized group did not again

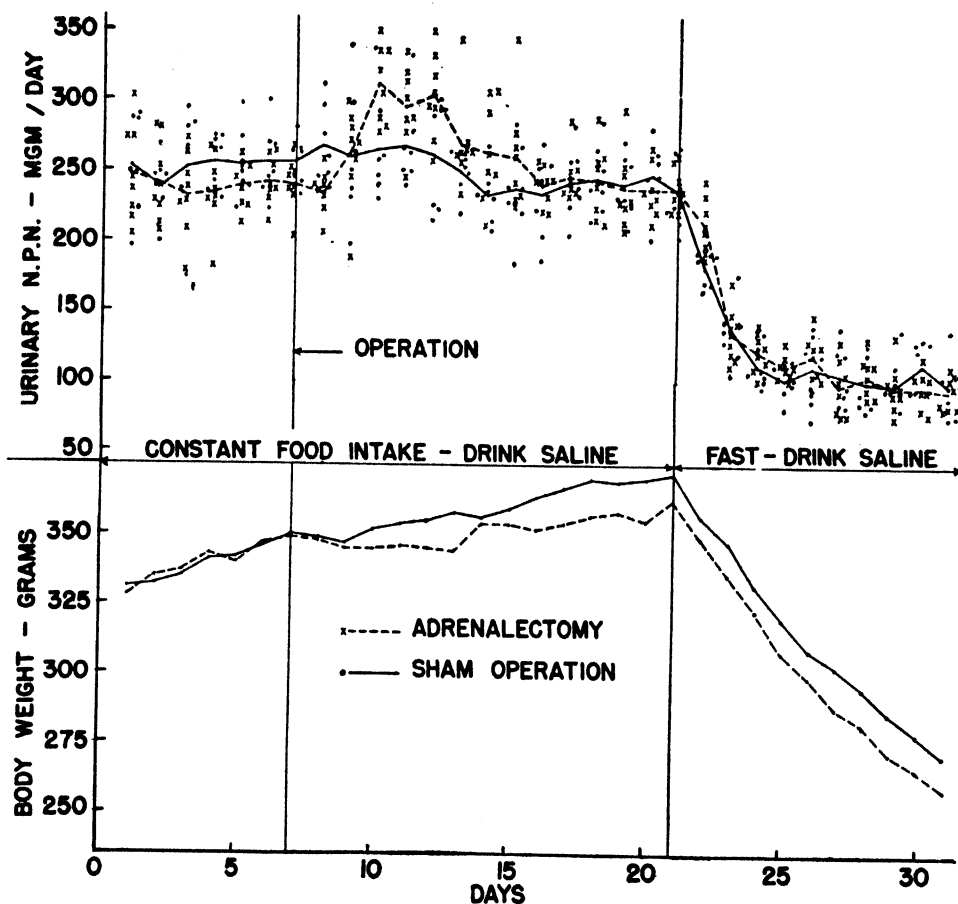


Fig. 1. Urinary non-protein nitrogens. Averages and individual values from rats fed a medium carbohydrate diet.

decrease significantly below its own pre-operative level or the level of the control group. During the 10-day fast the adrenalectomized animals excreted as much urinary nitrogen as their controls.

In experiment 2 (fig. 2), the medium protein diet was force-fed to 7 pairs of rats, and the other experimental conditions were identical with those of experiment 1. One rat developed symptoms of adrenal insufficiency and died during forced-feeding. The values for urinary nitrogen were decreased in this animal. The results from the remaining animals paralleled those of experiment 1 up to

the last 2 days of fasting when the level of urinary nitrogen of the sham-operated rats was definitely higher than that of the adrenalectomized rats.

In both experiments 1 and 2 (figs. 1 and 2), there was a tendency for the gains in weight of the adrenalectomized rats to be less than those of the controls. These differences may have been due to chance.

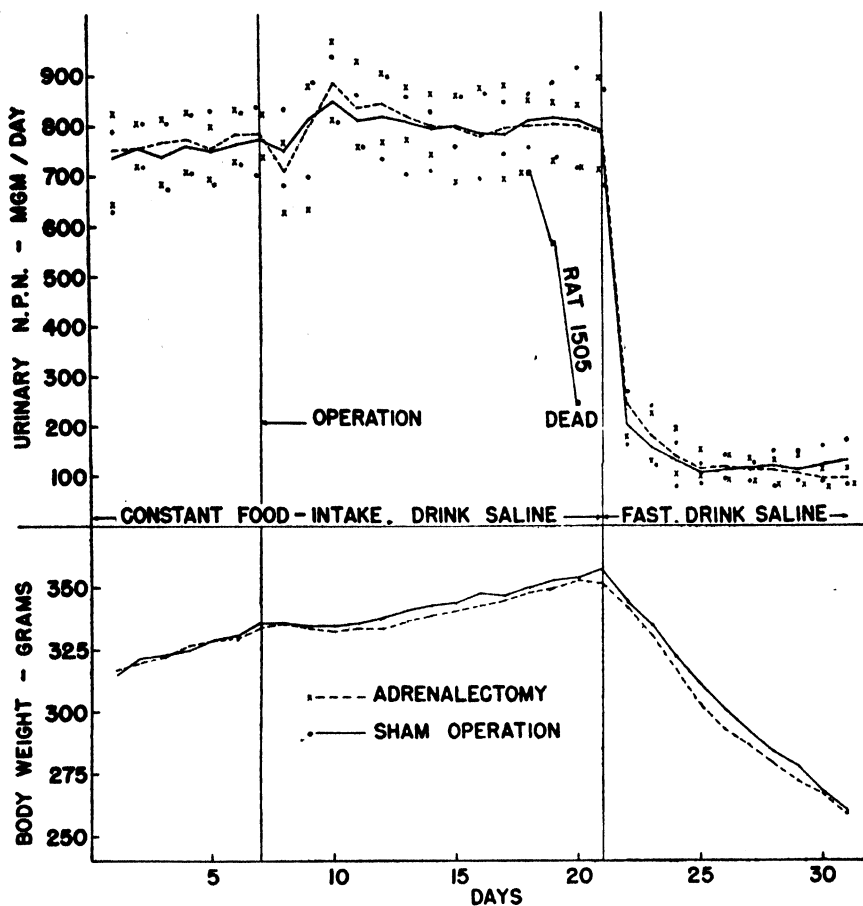


Fig. 2. Urinary non-protein nitrogen. Averages and range of values from 7 pairs of rats fed a medium protein diet.

In experiment 1 the average final weight of the thymus in the adrenalectomized group was 319 mgm., with a range of 223 to 421 mgm., whereas the regression of the thymus was more marked in the sham-operated group which had an average final weight of the thymus of 173 mgm., with a range of 134 to 251 mgm. Similarly, in experiment 2, the average final weight of the thymus in the adrenalectomized group was 286 mgm., with a range of 124 to 403 mgm., and the average in the sham-operated group was 141 mgm., with a range of 111 to 172 mgm.

In experiment 1 the values for blood glucose for the adrenalectomized group at the end of 10 days of fasting averaged 66 mgm. per cent, with a range of 60 to 73 mgm. per cent, and the sham-operated group had an average of 64 mgm. per cent, with a range of 45 to 76 mgm. per cent. In experiment 2, the final values for the adrenalectomized rats averaged 61 mgm. per cent, with a range of 47 to 85 mgm. per cent, and for the sham-operated group an average of 72 mgm. per cent, with a range of 54 to 86 mgm. per cent.

DISCUSSION. As far as the results of this study are concerned there was little evidence that the hormones of the adrenal cortex play a prepotent rôle in the catabolism of protein or in the ability of the animal to mobilize its own tissue proteins during fasting. In accord with all earlier work the weight of the thymus was decreased more rapidly by fasting in the presence of the adrenal

TABLE 1
Composition of fluid diets

CONSTITUENT	MEDIUM CARBOHYDRATE	MEDIUM PROTEIN
	grams	grams
Cellu flour (Chicago Dietetic Supply).....	120	120
Osborne and Mendel salt mixture.....	40	40
Dried yeast (Pabst).....	100	100
Wheat germ oil.....	10	10
Cod liver oil.....	10	10
Mazola oil.....	200	310
Vitamin K (2-methyl-1-4-naphthaquinone) ..	100 mgm.	100 mgm.
Casein (Labco).....	160	100
Starch.....	200	
Dextrin.....	190	
Sucrose.....	200	
Egg albumin (Merck).....		400
Water to make total volume of.....	2000 cc.	2000 cc.

glands than in their absence, but this difference was not reflected in the amounts of non-protein nitrogen excreted during fasting. The differences in the general results of these experiments and those of earlier studies (1) may be based upon differences in the conditions of the experiments, such as the forced-feeding of diets high in their content of fat and protein instead of carbohydrate, and in the continuation of the experiments over periods of several weeks instead of the usual acute experiments which have been completed in 1 or 2 days.

The medium protein diet (table 1) used in experiment 2 was free from carbohydrate except for the small amount present in the yeast. The good survival and vigor of these animals is not in agreement with the conclusion of Eversole (5) that the adrenal cortical hormones are essential for the prolonged survival of adrenalectomized rats fed a carbohydrate-low diet.

These data could be used to support the hypothesis that the defects in protein metabolism in the adrenalectomized animal are due entirely to secondary changes in the condition of the animal, such as were exhibited by the one animal which

developed symptoms of adrenal insufficiency (fig. 2) and died. On the other hand the superficiality of this study, although no more superficial than most related studies, should be emphasized. Nothing has been shown of the kinetics of protein catabolism or of the turnover of amino acids between the body fluids and tissues which may not be reflected by balance studies. Adrenalectomized, saline-treated rats may be incapable of metabolizing as great a load of dietary protein as normal animals and may be incapable of maximal mobilization of tissue proteins under more severe forms of stress than were tested in these experiments.

Positive evidence (1) of a relationship of adrenal cortical function to protein metabolism consists of the well established effect of the C-11 oxygenated adrenal cortical steroids in causing a breakdown of lymphoid and probably other tissues and inhibiting growth. Such evidence cannot be set aside by the negative data of the present study.

The question must be considered that the adrenalectomized animals used in this study were not actually deficient in cortical hormones due to the presence of accessory cortical tissue. This is highly improbable. During the past 6 years we have adrenalectomized many hundreds of rats and not one has survived indefinitely without the administration of hormones or saline, and not one has ever survived withdrawal of hormones or saline treatment, although each method of treatment has uniformly insured survival during its period of administration. Moreover, we have unpublished data showing that force-fed adrenalectomized rats uniformly develop symptoms of adrenal insufficiency and die following withdrawal of sodium chloride.

It was demonstrated by this study that a post-operative increase in the loss of nitrogen can occur in the absence of the adrenal hormones and that such animals are capable of mobilizing some of their own tissues during fasting. Our conclusions do not extend beyond stating that the rat has mechanisms regulating nitrogen balance, other than the adrenal cortical hormones, and that these adaptive mechanisms can function in the absence of the adrenal glands.

SUMMARY

In experiment 1 male rats were adapted to the forced-feeding of a medium carbohydrate diet for 2 weeks. The animals were given a solution of 1 per cent sodium chloride to drink during all phases of the experiment. Eight rats were adrenalectomized and 7 were sham-operated. During the first post-operative day the adrenalectomized rats excreted less nitrogen; but by the third day there was a rise in urinary nitrogen which was sustained for several days and was greater in the adrenalectomized rats than in their controls. During 2 weeks of post-operative feeding the urinary nitrogen of the adrenalectomized group did not decrease significantly below the pre-operative level or that of the control group. During a 10-day fast the adrenalectomized animals excreted as much urinary nitrogen as their controls.

In experiment 2 a medium protein diet was force-fed to 7 pairs of rats, but the other experimental conditions were identical with those of experiment 1.

The results paralleled those of experiment 1 up to the last 2 days of fasting when the level of urinary nitrogen of the sham-operated rats was definitely higher than that of the adrenalectomized rats.

Although it is not concluded from these experiments that protein metabolism is normal in adrenalectomized salt-treated rats, it is evident that the rat has mechanisms regulating nitrogen balance other than the adrenal cortical hormones and that adjustments in the metabolism of proteins can occur in the absence of the adrenal glands.

REFERENCES

- (1) INGLE, D. J. The chemistry and physiology of hormones. Publication of the American Association for the Advancement of Science, 83, 1945.
- (2) REINECKE, R. M., H. A. BALL AND L. T. SAMUELS. Proc. Soc. Exper. Biol. and Med., 41: 44, 1939.
- (3) INGLE, D. J. AND J. Q. GRIFFITH. Chapter 16, The rat in laboratory investigation. J. B. Lippincott Co., Philadelphia, 1942.
- (4) MILLER, B. F. AND D. D. VANSLYKE. J. Biol. Chem. 114: 583, 1936.
- (5) EVERSOLE, W. J. Endocrinology 37: 450, 1946.

THE EFFECT OF FASTING UPON THE TOLERANCE OF NORMAL RATS TO A HIGH CARBOHYDRATE DIET ADMINISTERED BY STOMACH TUBE

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During fasting the tolerance of animals to glucose and their ability to assimilate other forms of carbohydrate are decreased below normal. These and collateral changes which occur during fasting have been reviewed by Chambers (1938) and by Peters (1945). The production of glycosuria by administering carbohydrate to intact dogs at the termination of a fast was first described by Lehmann (1874). Hofmeister (1890) performed the classical quantitative studies on this condition which he termed "hunger diabetes." The purpose of the present study was to determine the effect of different periods of fasting upon the ability of intact male rats to assimilate a standard high carbohydrate diet administered by forced-feeding.

METHODS. Male rats of the Sprague-Dawley strain having initial weights of 300 to 315 grams were used. The animals were maintained on Purina Dog Chow until the beginning of fasting. The high carbohydrate fluid diet was made according to table 1. The diet and methods of forced-feeding were modifications of those described by Reinecke, Ball and Samuels (1939). Twenty-six cubic centimeters of this diet per day are required to sustain a normal rate of growth and to meet the caloric requirements of normal male rats weighing 300 grams.

The rats were fasted for periods of 1, 2, 7, 10 or 14 days. They were allowed water to drink ad libitum except for rats fasted 14 days which were given 1 per cent saline during the last 7 days of fast. At the end of the fasting period each animal was placed in an individual metabolism cage and 24-hour specimens of urine were collected. During the test period the high carbohydrate diet was fed to the groups each morning between 8:45 and 9:15 a.m., and afternoons between 4:45 and 5:15 p.m. The maximum amount of diet was determined which was tolerated by at least 7 of a group of 9 to 10 rats without the production of glycosuria. The feeding was continued for 3 days in those animals which did not exhibit glycosuria, and in the remaining animals until the glycosuria had disappeared or death had occurred. Each rat was used for only one test.

A mixture of sodium hydroxide and bismuth oxychloride (Denver Chemical Manufacturing Company) was used for qualitative detection of glucose in urine, and the Benedict titration (1911) for quantitative determination. Blood sugar was determined by the method of Miller and Van Slyke (1936).

RESULTS. The data showing the amounts of glucose excreted in relation to

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the daily food intake after different periods of fasting are summarized in figures 1 and 2. The average total glucose excretion per rat throughout the test period was considered a rough index of carbohydrate tolerance for those groups in which

TABLE 1
The high carbohydrate diet

	grams
Cellu-flour (Chicago Dietetic Supply)	120
Dried yeast (Pabst)	100
Egg albumin (Merck)	160
Salt mixture (Osborne and Mendel)	40
Cod liver oil	10
Wheat germ oil	10
Corn oil containing 100 mgm. vitamin K	10
Sucrose	250
Starch	500
Dextrin	250
Water to make total volume of	2000 cc.

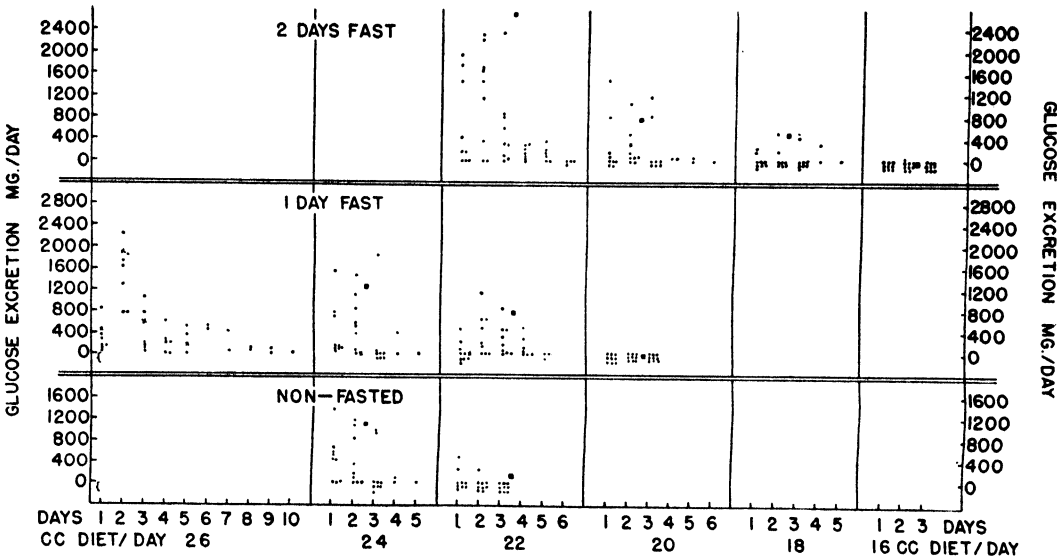


Fig. 1. The effect of fasting 1 or 2 days upon the glucose excretion following the administration of graded doses of a high carbohydrate diet.

× Animal died
□ The average total glucose excretion per rat of the group.

most of the rats survived (figs. 1 and 2). An excretion of reducing substances below 40 mgm. per day was arbitrarily considered negative, since normal adapted animals may excrete up to 40 mgm. per day of reducing substances.

The tolerance of normal rats to the high carbohydrate diet as estimated by glycosuria was progressively decreased by fasting for periods up to 7 days. The

results indicate, but do not prove, that a fast of 10 days was more effective than one of 7 days in reducing the carbohydrate tolerance. Five of 11 rats in the group fasted 10 days excreted glucose when given an amount of diet which was tolerated without glycosuria by rats fasted for 7 days. Three of 6 rats fasted 2 weeks and then, given 12 cc. of diet per day, excreted 280, 650 and 740 mgm. of

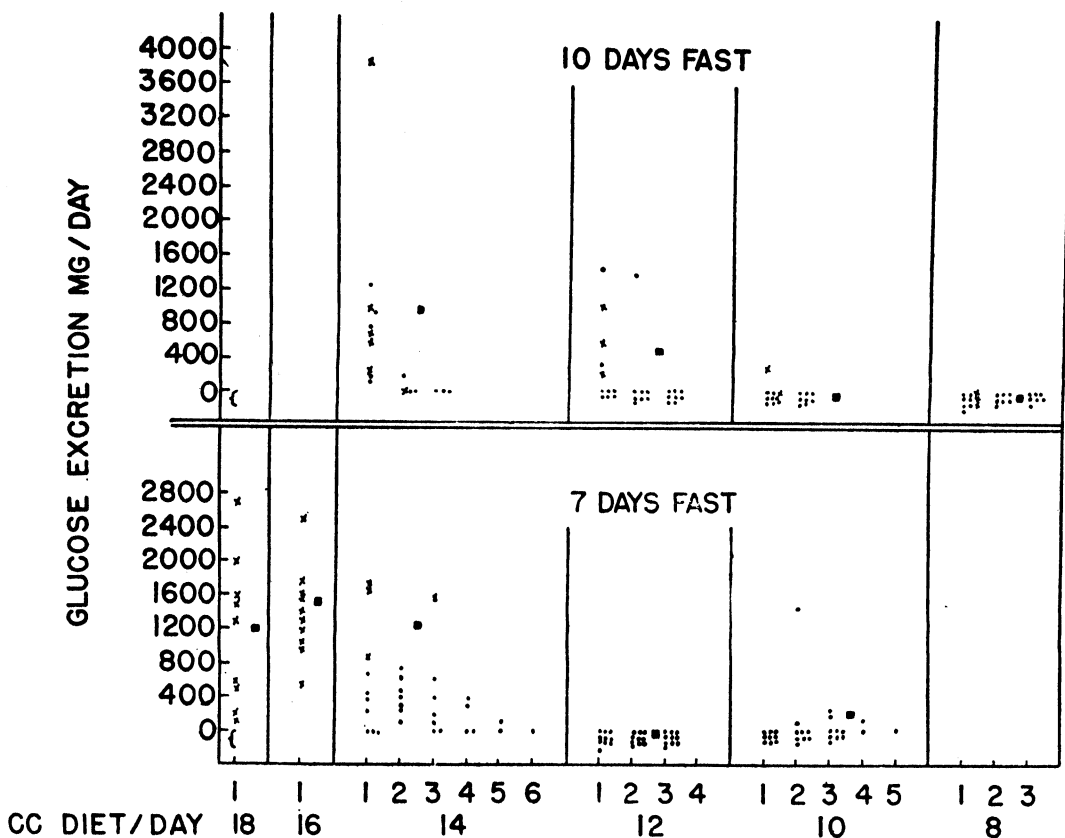


Fig. 2. The effect of fasting 7 or 10 days upon the glucose excretion following the administration of graded doses of a high carbohydrate diet.

× Animal died

□ The average total glucose excretion per rat of the group.

glucose, respectively, on the first day, while the other 3 excreted none. The series fasted 14 days was discontinued owing to the low incidence of survival.

A few blood glucose determinations were made which also showed a decrease in the ability of the animals to assimilate carbohydrate. Three rats fasted 7 days and then, given 2 feedings of 9, 8 or 7 cc. of diet each, died 32, 35 and 30 hours, respectively, following the first feeding. At this time the blood sugar levels were 570, 886 and 565 mgm. per 100 ml., respectively. They all had excreted more than 1.5 grams of glucose during the first 24 hours and had food present in the stomach at the time of death. In 3 rats fasted 7 days and then

fed 10 cc. of diet, blood glucose levels were elevated during the first 2 days but approached normal by the third to fourth day of feeding. Two of these rats had glycosuria, while the third did not.

When the intake of food exceeded the assimilation limit, disturbances other than those in carbohydrate tolerance were noted. Diarrhea and signs of dehydration were readily produced, and respiratory difficulties accompanied by an abundant nasal and oral mucous discharge were observed. In more severe cases the animals became lethargic. Convulsions occurred in some instances. The animals died, sometimes within 8 or 10 hours after the first feeding, in a condition resembling the shock which can be produced by a variety of other damaging agents. Ingle (1941) has referred to this syndrome as "food-shock." In animals dying of shock, pathological findings included hemorrhages and petechiae into the stomach wall and often into the stomach lumen. In the more severe cases practically the whole stomach wall was filled with extravasated blood. Occasionally intraperitoneal exudates containing varying amounts of blood cells were observed. Astwood, Flynn and Krayner (1942) noted intra-abdominal hemorrhages in dogs dying 3 to 25 days following infusions with relatively large doses of glucose which had been tolerated at the time of injection.

The survival rate of rats fasted more than 10 days was poor, although rats fasted up to 10 days remained vigorous. Most of the rats died after 11 to 13 days of fast. The few animals surviving to be tested after 14 days' fast represented those most resistant to the stress of fasting.

Numerous ulcers were almost invariably found in the cardiac portion of the stomach of rats fasted 1 week or longer. Erosions of the pyloric mucosa were not observed, but subepithelial hemorrhages occurred. No gross abnormalities were noted in the intestine. In rats fasted to the premortal or the fatal stage, long cords of deep, sometimes almost perforating, ulcers involved the whole cardiac mucosa. The loss of blood from the ulcerated mucosa was considerable, the intestinal lumina being filled with blood clots. In rats fasted 7 or even 10 days, ulcer formation was consistent with an apparently healthy condition. After 3 to 5 days of feeding, the ulcers were evidently rapidly closing and healing.

The animals dying from starvation usually showed hematuria about 24 to 36 hours premortally. In these the renal medullae were indistinguishable in color from the renal cortices, possibly because the medullary tubules contained the colored urine. The adrenals were hemorrhagic.

DISCUSSION. Fasting caused the expected decrease in the tolerance of intact rats to a force-fed high carbohydrate diet. The small amounts of food which fasted animals can tolerate are in sharp contrast to the tolerance which can be developed in similar animals by gradually increasing the daily intake of diet (Ingle, 1945). By this means, amounts as high as 41 cc. were tolerated before glycosuria developed, and much larger amounts (66 cc.) were given before death from "food-shock" resulted in all the animals.

The damaging effects of prolonged fasting are similar to those induced by

other damaging agents (Selye, 1940). Ulcer formation is one of the more difficult symptoms to elicit by other types of stress. The damage produced by fasting *per se* may, in a non-specific manner, limit the ability of the animals to assimilate the test diet and to withstand "food-shock." However, the tissue damage produced by fasting is not responsible for the syndrome leading to "food-shock," since the condition can be induced in non-fasted rats and in those fasted for short periods when tissue changes are not grossly evident. It is highly significant that, while the adapted animal can tolerate amounts of food much greater than that required for normal growth, the pre-fasted rat may die in "food-shock" from an intake of diet much smaller than the normal growth requirement for rats of similar initial weight.

The survival of fasted rats was given by Anderson (1943) as 23 days (range 22 to 25 days) for rats weighing 200 grams initially, and by Anderson and Joseph (1941) as 13 days (range 10 to 17 days). The strain of rats bred in Anatomy, College of Physicians and Surgeons, survived fasting for an average of 14.2 days (range 10 to 18.5 days). The initial weights of the latter animals were 300 to 400 grams. The survival time of the Sprague-Dawley rats used in the reported experiments was within this range also.

SUMMARY

Intact male rats were fasted for 1, 2, 7, 10 or 14 days, and then were forced a high carbohydrate diet. The tolerance to the diet was markedly decreased by fasting. Glycosuria, hyperglycemia and "food-shock" were produced by amounts of food which represented much less than a normal caloric intake for non-fasted rats of the same initial weight. The extent of reduction in the assimilation limit was related to the duration of fast.

The writer is very grateful to Dr. Dwight J. Ingle for his kind advice in carrying out this problem.

REFERENCES

- ANDERSON, E. Essays in biology. University of California Press, Berkeley, California. p. 33, 1943.
- ANDERSON, E. AND M. JOSEPH. Proc. Soc. Exper. Biol. and Med. **46**: 321, 1941.
- ASTWOOD, E. B., J. M. FLYNN AND O. KRAYER. J. Clin. Investigation **21**: 621, 1942.
- BENEDICT, F. G. J. A. M. A. **57**: 1193, 1911.
- CHAMBERS, W. O. Physiol. Rev. **18**: 248, 1938.
- HOFMEISTER, F. Arch. exper. Path. u. Pharmakol. **26**: 355, 1890.
- INGLE, D. J. Endocrinology **29**: 838, 1941.
- Endocrinology **37**: 488, 1945.
- LEHMANN, W. Arch. exper. Path. u. Pharmakol. **2**: 463, 1874.
- MILLER, B. F. AND D. D. VAN SLYKE. J. Biol. Chem. **114**: 583, 1936.
- PETERS, J. Yale J. Biol. and Med. **17**: 705, 1945.
- REINECKE, R. M., H. A. BALL AND L. T. SAMUELS. Proc. Soc. Exper. Biol. and Med. **41**: 44, 1939.
- SELYE, H. The Cyclopedia of Med. Surg. and Specialties **15**: 15, 1940.

THE INFLUENCE OF THIAMINE ON THE ACTION OF ACETYLCHOLINE ON MUSCLE¹

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The literature is replete with conflicting observations regarding the influence of thiamine on the action of acetylcholine on muscle, but the influence of hydrogen and other ions on this relationship has not been thoroughly studied. Decreasing acetylcholine reduces tonus of gastro-intestinal tract and thiamine deficiency causes anorexia. Bradycardia in thiamine deficiency was related to vagus effect which has been proved to be produced through acetylcholine (1). One group of workers (2) reported synergistic and another (3) antagonistic relations between thiamine and acetylcholine; some (4) reported both antagonism and synergism between thiamine and acetylcholine under different conditions, whereas others (5) asserted that no relationship exists between them. As these conflicting observations might be caused by a difference in the ionic and especially hydrogen ion concentration of the bathing fluid, a study was undertaken on changing the concentration of various ions, especially hydrogen ions in the perfusion fluid.

METHODS. Experiments were performed on *skeletal muscle*, *cardiac muscle* and *plain muscle of the intestine*. Regarding skeletal muscle, as it is convenient to study the effect of a drug on the onset of its fatigue and on its contraction-remainder, experiments were arranged to investigate the effects of different concentrations of thiamine alone, thiamine plus acetylcholine, and thiamine and acetylcholine with varying concentrations of calcium and potassium ions on the incidence of fatigue and the contraction-remainder caused by repeated contractions of a toad's gastrocnemius-sciatic preparation placed in a Keith-Lucas muscle chamber, and subjected to repetitive stimuli of constant intensity. The contractions were, as usual, recorded on a drum moving slowly at constant speed.

Regarding cardiac muscle, the heart of toads was perfused by the method of Basu (6). As thiamine is rapidly destroyed at pH 7.4, which is maintained during perfusion, its solution was introduced slowly by injection with a serum syringe into the tube leading to the heart cannula, so that the vitamin may not remain long in contact with the solution before reaching the heart.

Regarding plain muscle, the movements of loops of cat's intestine were recorded with Dale's apparatus. Fleisch's solution was used and its *pH* was

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kept constant at 7.6. The solution was regularly aerated rather than bubbling oxygen through it.

RESULTS. Direct effect of thiamine is negligible.

Acetylcholine hastens fatigue of skeletal muscle. The contraction-remainder of acetylcholine (3×10^{-8}) on skeletal muscle is completely inhibited by thiamine (3 mgm. per cent) (fig. 1). At a lower pH (6.8) the effect of thiamine is less marked. The acetylcholine-like action of acetates is much less pronounced than is expected from the law of mass action. The effect on the toad's heart is similar to that on skeletal muscle. The depressant action of acetylcholine on heart is completely annulled by thiamine (fig. 2). Thiamine cannot exert

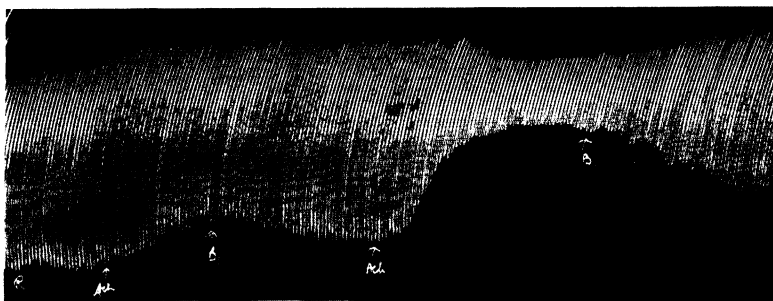


Fig. 1. Illustrating the action of acetylcholine (3×10^{-8}) on skeletal muscle and inhibition of thiamine (3 mgm. per cent) effect.

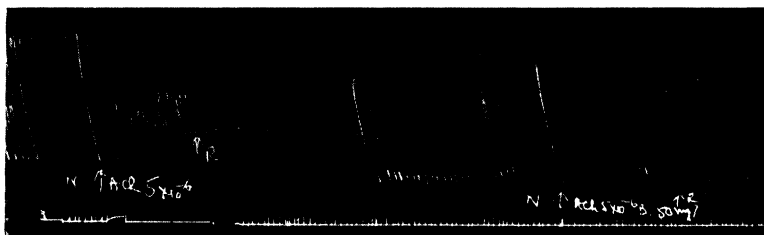
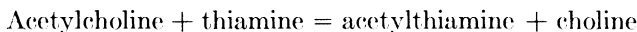


Fig. 2. Illustrating the action of acetylcholine (5×10^{-6}) on toad's heart and annulment of thiamine (50 mgm. per cent) effect.

this action properly in calcium-deficient Ringer's solution. Thiamine (25 mgm. per cent) has been shown to neutralize to a great extent the inhibitory effect of vagus stimulation on heart (fig. 3). Thiamine can partly annul the effect of acetylcholine on eserinated heart. Acetylcholine (1×10^{-7}) causes marked contraction of cat's intestine and addition of thiamine (5 mgm. per cent) brings about immediate relaxation.

DISCUSSION. Acetylcholine, supplied or produced at nerve terminals by nerve stimulation, accelerates decomposition of adenosine triphosphate (7), thus intensifying muscle contraction and leading to quicker fatigue. The inhibition of acetylcholine effect by thiamine is quite marked, not only on skeletal muscle, but also on the cardiac and intestinal muscles, although thiamine alone has but slight depressant effect on these muscles. The inhibition effect on acetylcholine is pronounced at a low concentration of thiamine (2 mgm. per

cent) at pH 7.4, but at lower pH levels concentration of thiamine needs to be raised. The negligible direct effect of thiamine on muscles indicates that inhibition effect on acetylcholine is produced by combining with, or otherwise modifying the radicle of acetylcholine, as shown by the following diagrammatic equation:



This conclusion is confirmed by the presence of a common acetyl radicle in acetylcholine and sodium acetate and by the experiment which shows the acetylcholine-like action of sodium acetate³. Thiamine appears to compete with choline for the acetyl radicle leading to formation of acetylthiamine. The enzyme, acetyl-thiaminase (8), in tissues may liberate thiamine back from acetylthiamine so formed. Thus acetylcholine may be destroyed either by

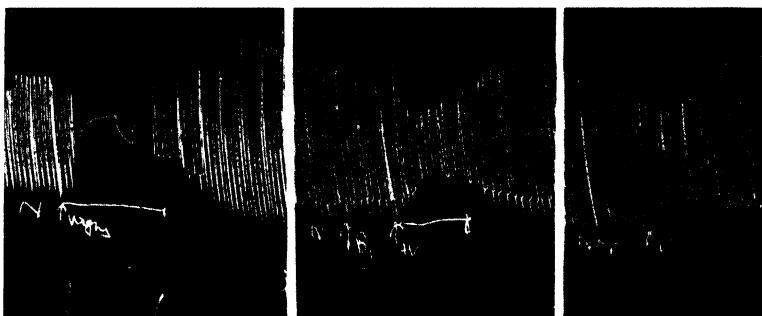


Fig. 3. Illustrating the influence of vagus stimulation on toad's heart and counteracting thiamine (25 mgm. per cent) effect on stimulation.

choline-esterase or by thiamine or by both. Thiamine may catalyse oxidation of acetic acid liberated from acetylcholine (9).

Most of the workers, who observed synergistic relations between thiamine and acetylcholine, worked on hearts of thiamine-low animals, at thiamine concentrations lower than that used in these experiments. In thiamine deficient animals, concentration of pyruvic acid in blood and tissues is high. If thiamine is supplied, it helps oxidation of pyruvic to acetic acid, which is used by the enzyme, cholineacetylase (11) to form acetylcholine. Acetylcholine thus formed intensifies the action of acetylcholine used in the experiments. We have used rather high concentration of thiamine (2 mgm. per cent and up) in normal animals which have a low content of pyruvic acid; so formation of acetic acid and acetylcholine is limited here; therefore synergetic action is not noted. As concentration of thiamine is high, there is interaction between it and acetylcholine.

In acid medium thiamine attaches acidic radicles and therefore cannot detach acetyl group from acetylcholine easily. Some observers did not observe reactions between thiamine and acetylcholine, because they did not work either with tissues of avitaminosed animals or with the high concentration of thiamine

³ The much less pronounced action of acetate can be explained from thermodynamical considerations (10).

required for demonstrating the antagonism between thiamine and acetylcholine at a suitable pH.

Thiamine has been shown to relax the contracted condition of muscle and is expected to help in sustenance of muscular performance if administered immediately before (12, 13), but not if added to diet (14), for in the former case, thiamine is in high concentration in the active muscle and can interact with acetylcholine and help oxidation of pyruvic acid.

The divergence of findings by various authors is due to the multiple reactions that may occur. Thus in thiamine-deficient animals, thiamine decarboxylates pyruvic to acetic acid, and probably by helping decarboxylation of serine to cholamine (which is methylated to choline), helps synthesis of acetylcholine. In normal animals, as concentration of pyruvic acid is low, thiamine cannot help synthesis of acetylcholine. So thiamine may participate either in synthesis or breakdown of acetylcholine with the help of either choline-acetylase or choline-esterase respectively, depending on the conditions.

SUMMARY

The action of acetylcholine on skeletal and cardiac muscles and strips of intestine has been recorded.

Acetylcholine accelerates onset of fatigue and delays relaxation of skeletal muscle. Thiamine was found to delay the onset of fatigue of a muscle both in Ringer's solution and in Ringer plus acetylcholine solution.

A study is reported on the influence of thiamine on the action of acetylcholine on cardiac, skeletal, and gut muscles. It was observed that thiamine in sufficiently high concentration annuls to various degrees the effect of acetylcholine.

An explanation has been suggested for this action of thiamine and experiments have been devised to support this explanation.

It was observed that thiamine does not annul the effect of acetylcholine when the calcium-ion level is too low. Thiamine in high concentration annuls the effect of acetylcholine on heart, even in presence of eserine. Thiamine has been found to prevent the inhibitory action of vagus stimulation on heart beats.

Acknowledgment. The author acknowledges his indebtedness to Prof. N. M. Basu for kindly suggesting and guiding him throughout the work.

REFERENCES

- (1) CARTER, C. W. AND A. N. DRURY. *J. Physiol.* **68**: i, 1929.
- (2) DICK, M. AND J. R. HEGE. *This Journal* **132**: 636, 1941.
- (3) BRECHT, K. AND S. MEINERS. *Pflüger's Arch.* **245**: 224, 1941.
- (4) JACKSON, B. AND G. WALD. *This Journal* **135**: 464, 1942.
- (5) MARTIN, J. AND K. LISSAK. *Arch. f. exper. Path. u. Pharmakol.* **198**: 667, 1941.
- (6) BASU, N. M. *Ind. J. Med. Res.* **28**: 405, 1940.
- (7) DUBOIS, K. P. AND V. R. POTTER. *J. Biol. Chem.* **148**: 451, 1943.
- (8) MASSART, L. AND R. DUFAYT. *Enzymologia* **8**: 392, 1940.
- (9) QUASTEL, J. H. AND D. M. WEBLEY. *Nature* **144**: 633, 1939.
- (10) CHAIKOFF, J. C. *Physiol. Rev.* **22**: 307, 1942.
- (11) NACHMANSOHN, D. AND H. B. STEINBACH. *J. Neurophysiol.* **6**: 397, 1943.
- (12) GOURNELLE, H. *Bull. et mém. Soc. méd. d. hôp. de Paris* **56**: 255, 1940.
- (13) MORELL, T. *Deutsch med. Wehnschr.* **66**: 398, 1940.
- (14) KEYS, A. AND A. F. HENSCHEL. *This Journal* **133**: 350, 1941.

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THE PERMEABILITY OF BLOOD CAPILLARY SPROUTS AND NEWLY FORMED BLOOD CAPILLARIES AS COMPARED TO THAT OF OLDER BLOOD CAPILLARIES

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The literature dealing with the permeability of blood capillaries is voluminous. Nothing appears to have been published, however, concerning the permeability of blood capillary sprouts and newly formed blood capillaries as compared to that of older capillaries. Yet this would seem to be a matter of some importance, since there now appears to be a general agreement (1) that the growth of the entire vascular system, after a brief period of primary differentiation, is accomplished by the sending out of capillary sprouts from pre-existing endothelium, and their anastomosis to form new blood capillaries. Not only are large numbers of blood capillary sprouts and new blood capillaries formed during all stages of growth, but also during the repair of wounds. If blood capillary sprouts and newly formed blood capillaries are more permeable than older capillaries, this would suggest that there may be a freer exchange of materials between the vascular system and the surrounding tissue in all conditions in which sprouts and newly formed capillaries are present in large numbers, such as during growth and during the earlier stages of wound healing.

MATERIAL AND METHODS. In the present experiments the permeability of blood capillary sprouts and newly formed blood capillaries, as compared to that of older capillaries, was studied by injecting the blue dye T. 1824¹ intravenously into rabbits having transparent moat chambers in their ears, and determining the time elapsing between injection of the dye and its appearance in the tissue outside of the sprouts and newly formed capillaries, and the older capillaries.

The moat chambers (2) used in these experiments are made of glass and mica, and each encloses a shallow space called the "bay." Following insertion of the chambers, vascularized tissue grows from the subcutaneous tissue of the ear into the bay through two entrance holes at one end. The bay has a glass bottom and a mica top and is only approximately 75 microns deep. Consequently the blood capillary sprouts, newly formed blood capillaries, and older capillaries in it can be seen distinctly with the microscope.

¹ The author is indebted to Dr. Magnus I. Gregersen for the T. 1824 used in these experiments.

The injections of dye were made, in all instances, when the vessels had grown about $\frac{3}{4}$ of the way across the bay. Since about three weeks are required for the tissue to grow this far, there were present in the bay at this time capillaries varying in ages from 1 day (at the growing periphery) to 21 days (in the region of the tissue first formed). The sprouts were in a state of active growth, and were forming new capillaries in the manner described by Clark and Clark (1), and at such a rate as to give rise, on the average, to a new plexus of capillaries approximately 100 microns in width every 24 hours. Capillary sprouts, newly formed capillaries, and older capillaries in tissue in a portion of the bay of a moat chamber are shown in figure 1.

The tissue was studied with the microscope before and after the injections at magnifications of 200 and 400 diameters, and was also photographed both before and after the injections. The film used in these experiments (panatomic X) did not show the distinct color differences produced by the dye. Therefore water color records were made to demonstrate the amounts of dye that passed through the walls of the sprouts, and newly formed capillaries, and the older capillaries, as indicated by the depth of color outside of these structures. The color of the dye in the tissues was observed directly with the microscope, and then matched as closely as possible with water color on drawing ocular tracings made just before injection of the dye. Such records were made one-half hour and four hours after injection of the dye.

Four rabbits were used, each with a chamber in one ear. In the case of rabbits 1, 2 and 3, 100 mgm./kgm. of body weight of dye were injected intravenously, via one of the lateral ear veins of the ear that did not contain the chamber. No dye was injected into rabbit 4. Thus the normal color of the tissue outside of the vessels in the chamber in this rabbit served as a control for changes in color of the tissues in the other chambers following injection of the dye.

The results secured with rabbit 1 will be described in detail; those secured with rabbits 2 and 3 were similar.

Since, as reported by Gregersen and Stewart (3), T. 1824 is not stable in saline unless some protein is also present, the dye was injected in 6 cc. of sterile distilled water in all instances.

OBSERVATIONS. Careful observations of the sprouts and capillaries were made before injection of the dye. In no instance was there any evidence of stickiness of the endothelium toward leukocytes, nor was there any indication that the permeability of the capillaries, or of the sprouts, was greater than normal.

Three minutes after injection of the dye the vessels were again examined with the microscope. At this time the dye was seen to be entirely confined within the vessels and sprouts. The color of the plasma was a clear blue, in contrast to the reddish-yellow color of the erythrocytes and the general pinkish hue of the tissue surrounding the sprouts and vessels.

The first traces of dye in the tissue were seen outside of the sprouts, adjacent to the youngest of the circulating capillaries, in the region marked zone 1 in figure 1. Within $\frac{1}{2}$ hour the dye was clearly visible in this region.

Within $1\frac{1}{2}$ hours after injection, the dye began to be visible outside of the newly

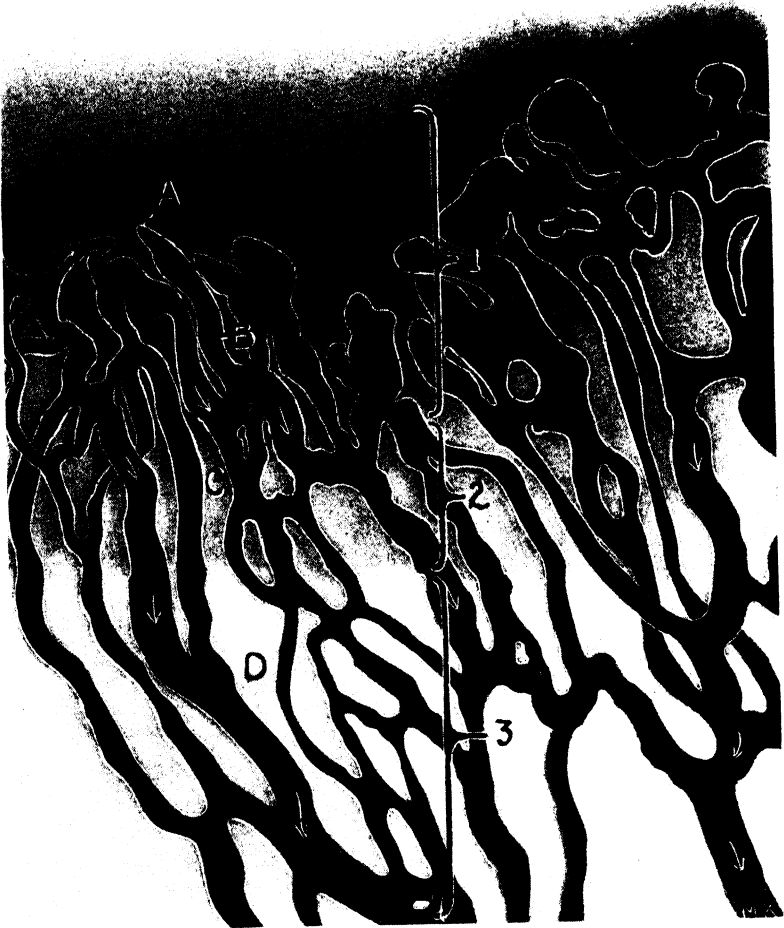


Fig. 1. Drawing ocular record of tissue in a part of the bay of the chamber in rabbit 1, showing blood capillary sprouts, newly formed blood capillaries, and older capillaries. This record was made 4 hours after intravenous injection of 100 mgm./kgm. of body weight of the dye T. 1824. *A*, tip of blood capillary sprout; *B*, side of blood capillary sprout; *C*, region of newly formed blood capillaries (approximately 24 hrs. old); *D*, region of older blood capillaries (approximately 48 hrs. old or older). This figure was made from a drawing in which the color of the dye was recorded with water color. The dark color within the sprouts and capillaries represents the blue dye, which at this time was still present within these structures in higher concentration than in the tissue outside of them. The different depths of color outside of the sprouts, the young capillaries, and the older capillaries, is due to differences in amount of dye that has passed through the walls of these structures into the surrounding tissue during the 4 hour period following its injection. Thus, as shown in zone 1, more dye has passed through the walls of the sprouts and the most recently formed row of circulating blood capillaries than elsewhere. As shown in zone 2 more dye has passed through the walls of the young blood capillaries than through the walls of the older ones in zone 3. $\times 200$

formed blood capillaries (not older than 24 hrs.) in the region marked zone 2 in figure 1.

Between 2 and 3 hours after the injection, dye began to be visible in the tissue outside of capillaries which were 48 hours old or older, including those in the tissue 3 weeks old.

Three hours after the injection blue granules could be seen in macrophages and leukocytes outside of the sprouts and in the connective tissue close to the most recently formed capillaries. Between these cells the dye, for the most part, was still distributed diffusely.

Four hours after the injection, three zones, based upon depth of color of the dye outside of the sprouts and circulating blood capillaries could be observed. In zone 1 the blue color was deepest. This was the region of the sprouts, and was adjacent to the peripheral side of the most recently formed row of blood capillaries. In zone 2 the blue color was paler than in zone 1. This was the region of recently formed blood capillaries (not older than 24 hrs.). In zone 3 the blue color was palest. This was the region of capillaries 48 hours old and older. These zones are shown in figure 1.

Each of the color changes noted above was checked by comparison with the color of the tissue in the control chamber in rabbit 4, into which no dye was injected. No color changes such as seen in the experimental chambers could be detected in the control chamber.

DISCUSSION. The above observations demonstrate that in the present experiments the blue dye T. 1824 passed more rapidly through the walls of the blood capillary sprouts and the most recently formed of the circulating blood capillaries than through those of the older capillaries (48 hrs. old or older).

Following its injection the dye could be detected first in the region of the sprouts (in zone 1 of fig. 1). It is not felt, however, that this is conclusive evidence that the sprouts were more permeable than the most recently formed of the circulating capillaries, since some of the dye may have passed into this region from the adjacent row of most recently formed capillaries. Therefore it is concluded only that the sprouts and most recently formed capillaries are more permeable than the older capillaries.

The present observations are in accord with studies of the permeability of blood capillaries during wound healing reported by Lange (4). This investigator injected the dye fluorescein intravenously and observed the amount of dye that appeared per unit time in surgical wounds during the process of healing. Using the dermofluorometer (5) for recording the dye concentrations in the tissue, it was found that 4 to 5 times as much dye appeared in the growing tissue of healing wounds per unit of time as in other regions of the body surface. That this was not due to the dye contained within the blood capillaries, which are increased in number during the early stages of wound healing, but to dye in the tissue outside of the capillaries, was shown by the fact that pressure upon the tissue with a glass slide, which forced the blood out of the capillaries, did not appreciably alter the readings. Subsequent measurements showed that the amount of dye appearing in the area of the wound, per unit time, returned to normal within approximately 2 or 3 months.

A question that must be answered in regard to the present findings is whether or not in the chambers used in these experiments the tissue surrounding the older capillaries was supplied with lymphatics, whereas that surrounding the newly formed capillaries was not. If this were the case, the dye might have accumulated to a greater extent outside of the newly formed capillaries due to lack of lymphatic drainage rather than to these capillaries being more permeable than the older ones. There are three reasons for thinking that this was not the case. In the first place, lymphatics frequently fail to grow into moat chambers, and in one of the chambers used in these experiments, a careful microscopic examination failed to show that any were present. Yet in this chamber, as in the others, the color of the dye outside of the newly formed capillaries became deeper than that outside of the older ones. In the second place, even when lymphatics do grow into moat chambers, they are comparatively few in number, usually not more than 3 or 4, so that much of the tissue in the chamber (even the older tissue) is not supplied by them. Yet no differences in depth of color surrounding the older capillaries were seen in different parts of the chambers. In the third place, even when lymphatics are present in moat chambers there is very little flow of lymph through them. Thus, as in the case with lymphatics in round table chambers (6), when red blood cells or white blood cells get into the lymphatics, they can often be seen bobbing back and forth in the same place for hours. It is not to be expected that such an inadequate flow of lymph would be very effective in removing dye from the tissue.

In view of the above considerations, it is felt that the paler color of the dye outside of the older capillaries was not due to removal of the dye from the tissue by the lymphatics, but to less dye passing through the walls of the older capillaries than through those of the younger ones.

SUMMARY

The permeability of blood capillary sprouts and newly formed blood capillaries, as compared to that of older blood capillaries, was studied by direct microscopic observation, using transparent moat chambers in rabbits' ears. These studies show that in such chambers the blue dye T. 1824, injected intravenously, passes more rapidly through the walls of blood capillary sprouts and newly formed blood capillaries than through the walls of older blood capillaries.

Thus the permeability of blood capillary endothelium was found to vary with age, the more recently formed endothelium being more permeable.

REFERENCES

- (1) CLARK, E. R. AND E. L. CLARK. *Am. J. Anat.* **64**: 251, 1939.
- (2) ABELL, R. G. AND E. R. CLARK. *Anat. Rec.* **60**: 161, 1932.
- (3) GREGERSEN, M. I. AND J. D. STEWART. *This Journal* **125**: 142, 1939.
- (4) LANGE, K. Personal communication, January 2, 1946.
- (5) LANGE, K. *Arch. Int. Med.* **74**: 175, 1944.
- (6) CLARK, E. R. AND E. L. CLARK. *Am. J. Anat.* **52**: 273, 1933.

THE NATURE OF INTRAPERITONEAL AND INTRARECTAL PRESSURES

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A series of experiments have been conducted at the School of Aviation Medicine, Randolph Field, Texas, for the purpose of exploring the physical characteristics of the pressures within the abdominal cavity. These studies were undertaken in the belief that such pressures might be of importance in opposing pooling of blood within the splanchnic circulation in man in the erect position and during exposure to positive radial acceleration. A comparison is made between observations made of pressures within the peritoneal cavity of dogs and intrarectal pressures in man.

A great deal of controversy has appeared in the literature concerning the magnitude of pressures recorded within the abdomen. Emerson (1) reviewed the literature prior to 1911. He was somewhat vague as to both the methods used and the results obtained in many instances, but interpretation of his remarks indicates that ten authors were cited who found atmospheric or subatmospheric pressures within the abdomen, and eighteen authors who recorded pressures which were positive or varied with changes in position. In general, positive pressures were observed when the experiments were conducted on subjects or animals in the erect position using balloons within hollow viscera in the pelvis or within the peritoneal cavity. Measurements made with human subjects or animals in the supine position using needles thrust into the peritoneal cavity were generally negative especially in the epigastrium. Wagoner (2) demonstrated negative pressures in the epigastrium of dogs in the supine position by means of a needle connected to a U-tube water manometer and also by observing the aspiration of fluid into the peritoneal cavity through a needle connected to a reservoir.

Overholt (3) and Lam (4) attributed the discrepancies mentioned above to the fact that a needle thrust into the peritoneal cavity and connected to a pressure recording manometer could not measure a positive pressure in the absence of ascites or pneumoperitoneum because under normal conditions there is nothing between the layers of visceral peritoneum to enter the needle. In other words, positive pressure can be recorded in a U-tube manometer for example only when air or fluid enters the needle, displacing the fluid in the U-tube. They attributed the variation in recorded pressures at various levels within the abdomen, and in various positions of the body, to the fact that the intra-abdominal pressure is a hydrostatic type of pressure. This conclusion was based on a consideration of the physical characteristics of the abdominal cavity and its contents and was substantiated by measurements of pressure at various levels in the abdomen and

with changes in the attitude of the body. No reports have been found in the literature dealing with the relationship between the magnitude of the pressures at various levels in the abdomen and height of the hydrostatic column of abdominal contents above the point of measurement.

If the intra-abdominal pressure is in fact a hydrostatic type of pressure, it should be possible to demonstrate a close relationship between the pressure at any level within the abdomen and the vertical height of the column of abdominal contents above the point of measurement. In the remainder of this report the term hydrostatic pressure or column is used to refer to the head of pressure provided by the mass of the intraperitoneal viscera.

I. INTRAPERITONEAL PRESSURE IN DOGS SUPPORTED IN THE ERECT POSITION. To study the relationship between the height of the column of abdominal contents and the pressure at various levels within the abdomen, sixty determinations of intraperitoneal pressure were made on six dogs. In each case the dogs received a 5 per cent solution of nembutal intravenously until a state of surgical anesthesia had been reached. Simple fluid manometers containing Skiodan (sp. gr. 1.260) were connected to small bore brass tubes. A rubber balloon, having a capacity of 7 to 10 cc. of air without an increase in internal pressure, was fastened over the end of each tube. These balloons were inserted into the peritoneal cavity through surgical incisions, which were then closed and rendered airtight. It was found that a great deal of care was needed to prevent the introduction of large quantities of air into the peritoneal cavity during the insertion of the balloons. A large syringe was inserted into the lower abdomen with the animal in the head down position to facilitate removal of intraperitoneal air. Several experiments were discarded because an excessive amount of intraperitoneal air remained undetected until after the experiment was completed. In four animals two balloons were inserted into the peritoneal cavity, one in the upper abdomen, the other just above the symphysis pubis. Variation in the height of the hydrostatic column of abdominal contents above the point of measurement was obtained by inserting the balloons at different levels and as a result of respiratory activity and differences in size of the experimental animals.

When these procedures had been completed, the animals were supported in the vertical position by tapes fastened to the upper extremities. The manometer tubes were arranged alongside the animal in the mid-axillary line and a cassette holder containing a 14 inch x 17 inch x-ray film was placed in a special rack immediately behind the animal. The balloons were inflated by introducing into the tube connecting the balloon and the manometer, a quantity of air sufficient to partially inflate the balloon. Proper inflation was checked by noting the appearance of changes in pressure coincidental with respiratory activity. If the balloon was not folded, an additional 5 cc. of air could be inserted into the system with no change in the average level of the meniscus in the manometer. Folds in the balloon were eliminated by applying a pressure of 40 or 50 cm. of fluid and manipulating the brass tube, then evacuating the balloon and refilling it with the proper amount of air. Roentgenographic exposures were made from a distance of 72 inches so that the diaphragm, the balloons within the abdomen, and the pressure manometers, were visible on a single roentgenographic plate (fig. 1A).

Simultaneous measurements of pressure from balloons within the abdomen and within the rectum were attempted and abandoned because relaxation of the anal sphincter due to the anesthesia resulted in the prompt evacuation of the rectal balloon. It was felt that fixation of the balloon in place within the rectum might result in excessively high readings.

The respiratory activity of the animals was considerably altered by the depth of the anesthesia and apparently, to a certain extent, as a result of being suspended in the erect position. The inspiratory efforts were of brief duration while the expiratory phase was prolonged and followed by a long pause. The pressures within the manometers gradually increased during inspiration and dropped abruptly at the beginning of expiration. Roentgenographic exposures were made in all phases of the respiratory cycle.

On each roentgenographic plate, measurements were made of the distance from each balloon to the highest point on the dome of the diaphragm and the height of the column of fluid within the manometer registering the corresponding intra-abdominal pressure. The distance from the balloon to the dome of the diaphragm was considered to be the height of the hydrostatic column of the intra-abdominal contents. The pressures within the manometers represented the effective pressure present at the level at which the measurement was taken. These pressures were corrected to a specific gravity of 1.050, approximately the specific gravity of blood, to facilitate comparisons between the intra-abdominal pressure and the estimated venous pressure at the various levels within the abdomen.

Results. Plotting the pressure within the abdomen, at various levels below the diaphragm, against the height of the hydrostatic column above the point of measurement reveals that there is a close relationship between these two variables (fig. 1B). The correlation coefficient between the intra-abdominal pressure and the height of the hydrostatic column was 0.834 (when n is 43, r 0.01 equals 0.418). This correlation is unexpectedly good considering the fact that differences in the state of anesthesia and tonus of the skeletal musculature plus the variation in pressure that occurs with respiratory activity, all would tend to reduce the correlation between these two factors.

The regression coefficient of the height of the hydrostatic column on the intra-abdominal pressure (fig. 1B regression line A) was 0.56. The regression of the intra-abdominal pressure on the height of the hydrostatic column (fig. 1B regression line B) was 1.23 and its reciprocal was 0.81. When the intra-abdominal pressure was calculated in terms of a column of fluid having a specific gravity of 1.00, the effective specific gravity of the organs within the peritoneal cavity was found to lie between 0.59 and 0.85. Extrapolation of these regression lines revealed that at the point where the height of the hydrostatic column was zero, the pressure within the abdomen should be somewhere between 4.35 and 0.60 cm. of fluid (sp. gr. 1.050). A positive pressure at the top of a hydrostatic column suggests an external source of pressure such as the tonus of the skeletal musculature of the abdominal wall and diaphragm. It is not considered likely that excessive inflation of the intra-abdominal balloons was responsible for this

finding inasmuch as considerable care was taken to avoid exceeding the capacity of the balloons during the experimental procedures. The average difference between the vertical distance from the balloons in the lower abdomen to the highest point on the dome of the diaphragm and the intra-abdominal pressure (cm. fluid sp. gr. 1.050) was 4.3 cm. Determinations in the upper abdomen revealed that intra-abdominal pressure as measured in the manometer was approximately equal to the distance from the balloon to the diaphragm. From an average of nineteen determinations, it was found that the pressure in the balloon was 0.78 cm. of fluid greater than the linear distance from the balloon to the diaphragm.

II. INTRARECTAL PRESSURES IN MAN. Measurement of the intra-abdominal pressure in man presents a more formidable problem than in experimental animals. Recording pressure from balloons placed within the hollow viscera in the

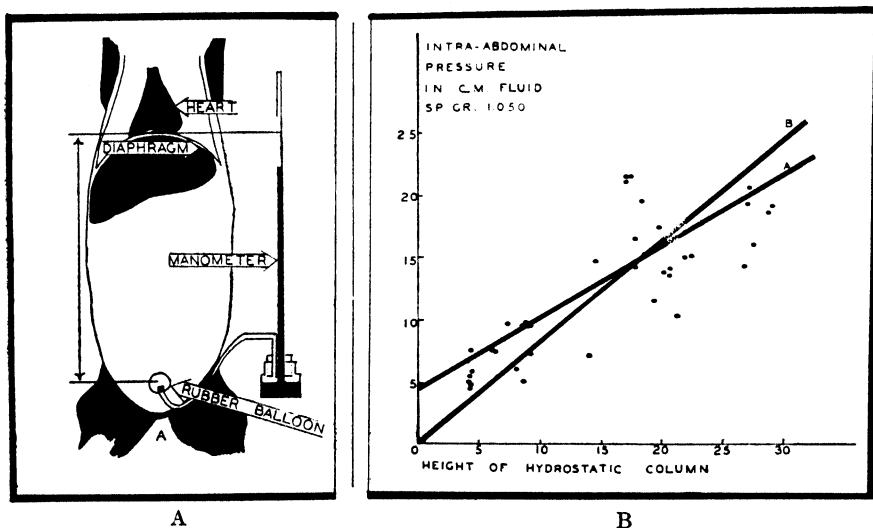


Fig. 1. Intraperitoneal pressure measurements in dogs

abdomen has been looked on with disfavor. However, if the pressure within the abdomen is a hydrostatic pressure, insertion of an air-containing balloon within the rectum of an individual should record a pressure equal to the sum of the following contributory pressures: *a*, the hydrostatic pressure head of the abdominal organs; *b*, pressure exerted by the smooth muscle in the walls of the rectum; *c*, pressure due to tonicity of the skeletal musculature of the abdominal wall and diaphragm.

In the belief that the first factor, *a*, might be the overwhelming contributor to measured intrarectal pressure, a series of fifty determinations of intrarectal pressure were obtained on five subjects in the horizontal position, tilted to 30° from the horizontal, 60° from the horizontal, and in the erect position (fig. 2A). In this way the effective height of the hydrostatic column of abdominal contents could be varied and the relative importance of this factor on the measured intra-

rectal pressure could be estimated. A technique similar to that described in Part I was used with the exception that the air-containing balloon was inserted into the ampulla of the rectum.

Due to difficulty encountered in consistently including an intrarectal balloon and the dome of the diaphragm on the same 14 inch x 17 inch plate, a framework was constructed which would support two 14 inch x 17 inch x-ray plates end to end with their long axes parallel to the long axis of the body. A section of sheet lead, having notches 1 cm. apart along one edge, was fastened across the space between the two x-ray plates on the surface of the cassettes. In this way the relative position of the two x-ray plates could be accurately reproduced during measurements which were made following development of the films. The framework was fastened rigidly to the edge of a manually operated, tilting x-ray table and the subjects reclined in the supine position on the table. The body of the subject was elevated from the surface of the table by means of a narrow wooden platform two inches high extending from head to heel to allow visualization of the brass tube and balloon within the rectum on the x-ray plate. A simple pressure recording manometer containing a fluid with a specific gravity of 1.26 was suspended in front of the cassettes in such a way that the manometer tube was always vertical. Two sets of roentgenograms were made in each position. Forty-four additional measurements were made on sixteen subjects in the erect position. In the experiments mentioned above, measurements of the vertical distance from the balloon to the top of the column of abdominal contents (fig. 2A) and the height of the column of fluid within the manometers were corrected for parallax resulting from divergence of the x-rays. The height of the column of fluid in the manometer was corrected to a specific gravity of 1.050 to facilitate comparison with the results on experimental animals described in Part I.

Results. The pressures within the rectal balloon were plotted against the corresponding distances from the balloon to the dome of the diaphragm (fig. 2B). The correlation coefficient was found to be 0.882 (when n is 50, r 0.01 equals 0.380). The regression coefficient of the height of the hydrostatic column on the intra-abdominal pressure (fig. 2B regression line A) was 0.63. The regression of the intra-abdominal pressure on the height of the hydrostatic column (fig. 2B regression line B) was 1.25. The reciprocal of the regression of the height of the hydrostatic column on the intrarectal pressure was 0.80. In this case the estimated effective specific gravity of the abdominal contents should be between 0.66 and 0.84. Extrapolating along these regression lines reveals that if the height of the hydrostatic column were zero, the manometer pressure should be between 5.3 and 0.65 cm. of fluid (sp. gr. 1.050).

Forty-four determinations of the intrarectal pressure were plotted against the corresponding height of the hydrostatic column of intra-abdominal contents (fig. 2C). It is seen that the greatest variability is in intrarectal pressure where the range is from 19.5 cm. of fluid to 45.8 cm. while the range in the height of the hydrostatic column is from 30.2 to 40 cm. This is probably due primarily to observed changes in intrarectal pressure as a result of respiratory activity. The mean values for intrarectal pressure and the height of the hydrostatic col-

umn of abdominal contents are 29.7 and 34.4 cm., respectively. The average difference between the two is 4.7 cm.

At no time was there visible evidence of pressure changes in the manometer due to peristaltic activity of the rectal walls.

DISCUSSION. The data on the magnitude of the pressures within the peritoneal cavity in dogs appear to be consistent with the results reported by Lam (4). Direct comparisons cannot be made because no information was presented concerning the vertical height of the column of abdominal contents above the

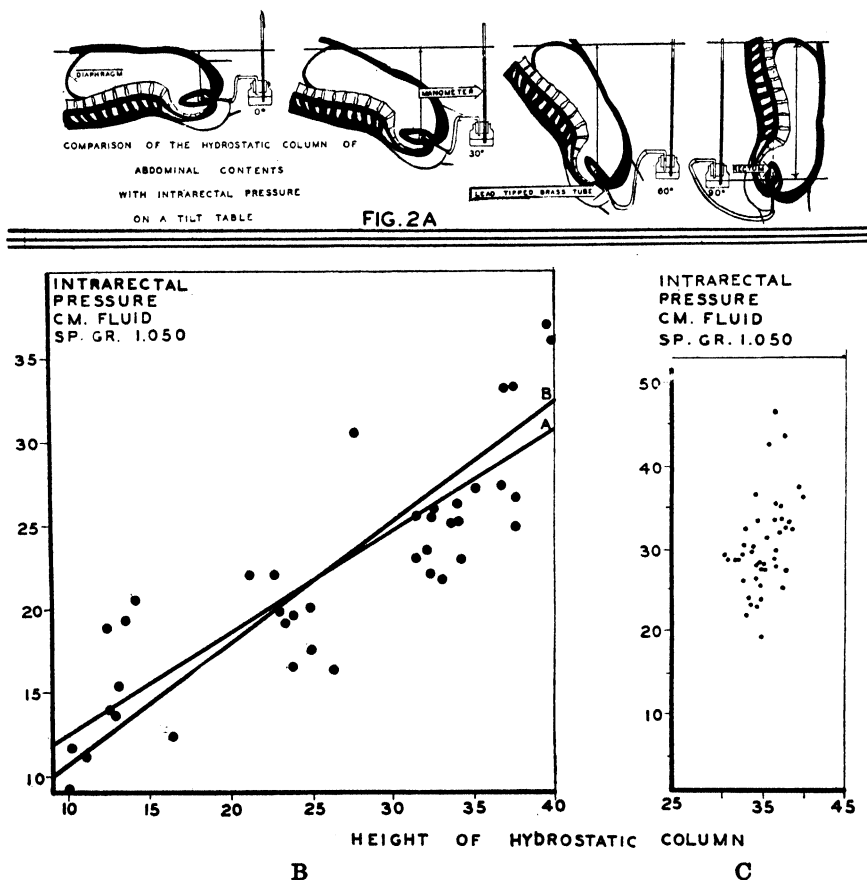


Fig. 2. Intrarectal pressure measurements in man

point of measurement. Measurements obtained by Mengert and Murphy (5, 6) from human subjects in the sitting position using intravaginal balloons are of the same order of magnitude as those reported above.

The correlation between intra-abdominal pressure at various levels within the abdomen and the vertical height of the column of abdominal contents above the point of measurement strongly suggests that the pressure within the abdomen is predominately a static pressure produced by the weight of the overlying abdominal organs.

In the experiments on dogs the measurements were all made in the erect position so the tonus of the abdominal musculature and diaphragm would not be expected to vary in relation to the levels at which determinations were made. On the other hand, the intrarectal pressures on man were obtained in the horizontal position with the abdomen flaccid and tilted 30° and 60° and in the erect position where the abdominal walls must support the weight of the abdominal contents. The fact that the difference in the regression coefficients under these two conditions is small suggests that the contribution provided by the tonus of the skeletal musculature of the anterior abdominal wall and diaphragm is small compared to the total recorded pressure as long as relaxation is maintained. This is substantiated by the fact that at the point where the hydrostatic column was zero, the intra-abdominal pressure was apparently less than 5.3 cm. fluid (sp. gr. 1.050).

Comparing the results obtained by measuring intraperitoneal pressure of dogs and intrarectal pressure of humans reveals a striking similarity in the correlation coefficients, regression coefficients and points of intercept of the regression lines on the ordinant. This strongly suggests that intrarectal pressure can be used as an indication of intraperitoneal pressure since the contribution of the rectal wall to the recorded pressure appears to be negligible for practical purposes.

Since the measurements of intrarectal and intra-abdominal pressures were obtained by means of air-filled balloons, pressures exerted on the outside of the wall of these balloons should correspond to pressures being exerted on the outside walls of blood vessels. Such pressures would tend to balance the hydrostatic pressure of the column of blood within the vascular elements of the splanchnic circulation particularly on the venous side. The venous pressure, for instance, is primarily the hydrostatic pressure of the column of blood extending from the point of measurement to the level of the right auricle. It is believed that pressure being supported by the vein wall is equal to the difference between the venous pressure and the intra-abdominal pressure at any particular level in the abdomen. Under these circumstances the average intra-abdominal pressure is approximately 5 cm. of blood less than the venous pressure in the lower abdomen and approximately equal to the intra-abdominal pressure in the upper abdomen. Since the intra-abdominal pressure tends to balance the venous pressure, the walls of the veins are required to support, by their own elasticity, only a small fraction of the venous pressure at any level within the abdomen. This is believed to have the effect of reducing the tendency for dilatation of the veins of the splanchnic circulation under the influence of gravity and, in this way, opposing the pooling of blood within the splanchnic veins and small blood vessels. As a result of these findings, the importance of the tonus of the skeletal musculature of the abdominal wall and diaphragm in supporting the splanchnic system is open to question.

There are two factors which complicate the picture to a certain extent. The contents of the abdominal cavity have not a homogeneous specific gravity, and the liver and spleen, having relatively large density, are held in place at the top

of the column when the body is in the erect position. The subatmospheric intrathoracic pressure, applied to the superior surface of the diaphragm, may affect the overall intra-abdominal pressure by an unknown amount. The specific effects of these two factors have not, as yet, been investigated. The observation that the effective specific gravity of the abdominal contents appears to be less than 1.000 requires additional investigation.

CONCLUSIONS

1. The pressure within the abdominal cavity is primarily a manifestation of the head of pressure provided by the mass of the movable organs within the peritoneal cavity. The magnitude of the recorded pressure is directly related to the vertical height of the column of abdominal contents above the level at which the measurement is made.

2. The tonus of the skeletal musculature apparently contributes a relatively small amount of pressure to the total recorded pressures if the subject remains relaxed in the erect position.

3. The intra-abdominal pressure may partially balance the venous and capillary pressures, opposing the tendency for pooling of blood within the splanchnic reservoir. The experimental results indicate that the average pressure supported by the venous walls is less than 5 mm. Hg at any level in the abdomen.

4. The determination of intrarectal pressure appears to be a reliable method of estimating variations in intraperitoneal pressure.

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REFERENCES

- (1) EMERSON, H. Arch. Int. Med. **7**: 754, 1911.
- (2) WAGONER, G. Am. J. M. Sc. **171**: 697, 1926.
- (3) OVERHOLT, R. H. Arch. Surg. **22**: 691, 1931.
- (4) LAM, C. R. Arch. Surg. **39**: 1006, 1939.
- (5) MURPHY, D. P. AND W. F. MENGERT. Surg., Gynec. and Obst. **57**: 487, 1933.
- (6) MENGERT, W. F. AND D. P. MURPHY. Surg., Gynec. and Obst. **57**: 745, 1933.

DESENSITIZATION OF MICE TO PLACENTAL TOXIN

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Toxic placental extracts can be assayed by intravenous injection into mice (1). However, the response to the toxin is not equal under all conditions, for, as found below, the sensitivity not only increases with age but the animals become refractory to the toxin following injection of sub-lethal doses.

METHOD. Simple saline extracts were prepared from placental tissue which was first perfused free of blood with saline. The placental cotyledons were stripped from the supporting structures and frozen until the time of extraction. The extraction was carried out by repeated comminution in saline with a "Waring blender." The combined supernatants which were obtained by centrifugation were frozen. When needed, the extracts were thawed, centrifuged, assayed and diluted to appropriate strengths with saline.

The unit of toxin, the MLD (Minimum Lethal Dose) was determined by interpolation between different doses injected intravenously into successive mice under ether anesthesia. The assay was facilitated by the prompt end point, death of the animals, within one to three minutes after the injection.

RESULTS. The toxin had the properties of a protein. It was inactivated by heating to 85°C. for ten minutes, and was retained by a cellophane membrane during dialysis against physiological salt solution. It was precipitated by half-saturated ammonium-sulfate solution or by fifteen per cent alcohol in the cold. With both methods there was a considerable loss of toxin. The toxin was poorly soluble in distilled water. It resuspended in physiological salt solution as a turbid preparation. According to nitrogen determinations, in the simple saline extracts, 0.10 mgm. of protein corresponded to one unit of toxin; for preparations reprecipitated with alcohol, 0.06 mgm. corresponded to one unit.

With the partially purified preparations, as with the simple saline extracts, intravenous injections were lethal to mice, sub-lethal doses resulted in focal liver necrosis, and the toxic extracts were inactivated by serum (2).

The data of figure 1 demonstrate the validity of the mouse assay to be within an error of approximately ten per cent. Dilutions were prepared from toxic extracts of 50 u/cc. The separate dilutions were then assayed on male mice of 18-21 grams. The MLV (Minimum Lethal Volume) for each dilution was plotted in figure 1. For MLV up to 0.40 cc., the MLV was directly proportional to the dilution of the extract.

Figure 2 shows the relation of the MLV (per mouse) to the body weight. The data were calculated from the curve of figure 3. From the curve it is to be seen that the dosage was not directly proportional to the body weight, but more nearly to its logarithm. Above 20 grams, the error would be small if no

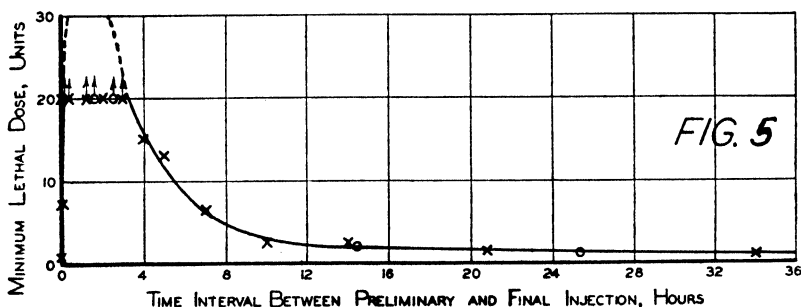
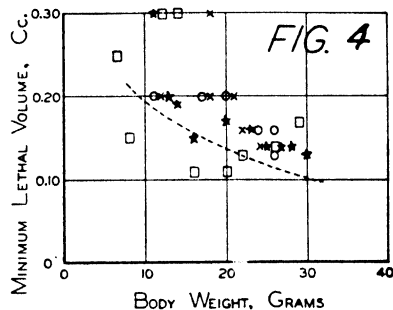
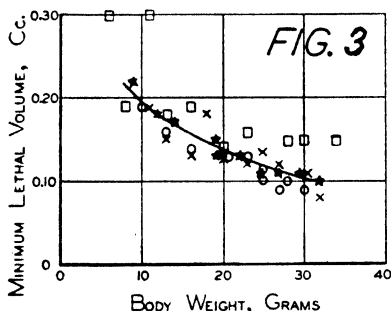
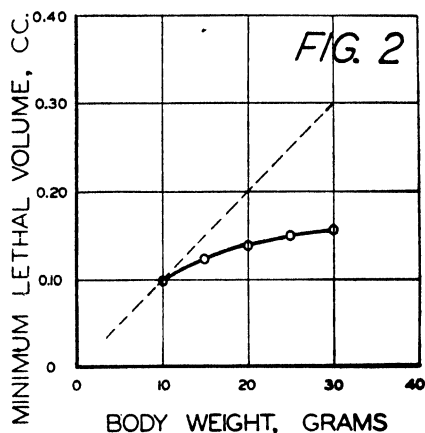
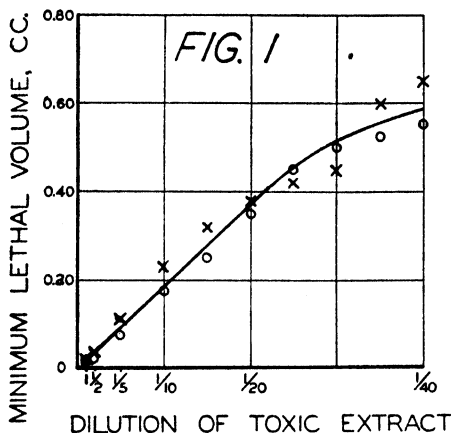


Fig. 1. The relation of the minimum lethal volume to the dilution of toxic extract. Circles, simple saline extract. Crosses, partially purified extract. (1, not diluted; $\frac{1}{2}$, diluted twice; $\frac{1}{5}$, diluted five times, etc.)

Fig. 2. The relation of the minimum lethal volume (per whole mouse) to the body weight.

Fig. 3. Minimum lethal volumes of toxic extract (per twenty grams of body weight) for mice of different body weights. The data of the two experiments with male mice are represented by crosses and stars respectively; the data for the two experiments with female mice by circles and squares.

Fig. 4. Minimum lethal volume of toxic extract at the time of secondary injections two days after the primary injections of figure 3. The broken line curve shows the curve of figure 3 for comparison. Symbols as in figure 3.

Fig. 5. Minimum lethal dose, in units, at different intervals after a primary sub-lethal injection. Crosses, male mice; circles, female mice.

correction at all were made for the body weight, hence this part of the curve supports Obata's (1) procedure of ignoring body weights. For the highest accuracy, mice of a standard weight, 20 grams, should be used.

In figure 3, in which the MLV of extract, per 20 grams of mouse, is plotted against total body weight, it is to be seen that the sensitivity, per 20 grams of mouse, increased with increasing body weight. This increase of sensitivity, per gram of body weight, was two-fold from the age of nursing (10 grams) to the age of full maturity (30 grams). It is clear that the sensitivities of male mice and of non-pregnant female mice were approximately equal at any given body weight.

Following these single intravenous injections of placental toxin, the sensitivity of the mice decreased. The extent of this desensitization or refractory state to the toxin can be seen in figure 5. The onset of the desensitization was rapid, but not instantaneous; the mice were already able to survive 7 units (7 MLD) at three minutes after the preliminary injection. Within 10 minutes they could withstand more than 20 units, i.e., enough to kill more than 20 ordinary mice having no pre-treatment. During the next three hours (fig. 3), the desensitization was so extensive that, with the exception of one assay, the lethal dose remained greater than 20 units. Some of the mice survived these massive doses with mild depression of activity, others passed through a temporary state of collapse.

Within 24 hours the sensitivity had returned almost to normal as can be seen from figure 5. The close approximation to normal sensitivity within two days is shown on a larger scale in figure 4; there it is to be seen that recovery to normal sensitivity was independent of the age (body weight). Male and non-pregnant female mice became refractory and returned to normal sensitivity in comparable ways (figs. 4 and 5). The sensitivity remained essentially constant after two days, the lethal dose being as high as two units at 15 and 20 days in one series, but remaining at one unit throughout other series. Tests which were run at 3, 4, 9, 10, 13, 15, 19, 20 and 23 days failed to show increase above normal sensitivity. In no case was less than one unit lethal; i.e., an anaphylactic type of sensitivity to the extracts could not be induced by a single injection.

The average level of inactivating potency in the bloods of five mice of 20 grams body weight, at 90 minutes after a sub-lethal injection of toxin, i.e., during the highly refractory period, was 31 units of inactivator per cc. of blood. The average of five untreated controls was 32 u/cc. Neither were inactivating titres of blood of mice 9, 13, 19 and 24 days after a sub-lethal injection significantly altered, the averages (3 animals) being 27, 27, 30, 30 u/cc. respectively; nor did the level of inactivator vary significantly with age, the values for nursing mice of 7 grams and for several months old mice of 24 grams being 28 and 29 u/cc. of blood.

The total incidence of grossly visible focal liver lesions was 18 per cent (of 152 animals). These lesions were not present in mice that were killed by the injection; they progressed from an engorged to an anemic stage.

Another focal liver lesion, probably of the type described by Olitsky and

Casals (3), occurred in some of the mice. These were pin-point, white spots which unlike the larger, irregular or reticular, induced lesions, showed no special predilection to the borders of the lobes. They occurred irrespective of injection of the toxin. They were present in about 21 per cent of the mice of more than 20 grams (i.e., more than approximately 50 days) and occurred occasionally in younger mice. Neither the lethal effect of the toxin, nor the incidence of induced lesions was markedly different in mice already having these naturally occurring lesions.

For the first few hours, newly induced lesions usually could be distinguished by their hemorrhagic appearance. Thus, usually, new lesions or newly superimposed lesions could be distinguished from two-day old lesions. The incidence of new lesions following the second injection was not markedly influenced by the presence of lesions which had been induced by the primary injection of two days earlier.

By contrast with the low incidence of focal liver lesions after the primary injections and by the secondary injections at two days, the incidence of focal lesions following injections of massive doses (20 units or more) during the refractory interval was above 95 per cent. These lesions were usually extensive. Bleeding into the small intestine was not uncommon.

The kidneys of some of the animals receiving massive second doses appeared contracted and pale at sacrifice; occasionally they were white and shrunken with a finely nodular surface. In a few instances there were also white streaks present in the heart muscle, diaphragm and occasionally elsewhere. Some of the animals with multiple lesions died after a few days.

Microscopically, the liver lesions were similar to the less frequent lesions that followed single injections. The kidney lesions were made up of extensive regions with markedly dilated tubules having a low cuboidal epithelium; there were many granular casts, particularly in the collecting tubules.

Extracts of mouse placentas were toxic to mice in the same manner as extracts of human placentas. Although not perfused, mouse placentas yielded 50 to 100 units of toxin per gram of placenta. These extracts were inactivated by mouse serum. In sub-lethal doses, the extracts resulted in a refractory period. Massive doses resulted in focal liver necrosis and kidney lesions.

DISCUSSION. Since the sensitivity of the mice could be varied experimentally by administration of preliminary sub-lethal doses of toxin, it is conceivable that the increase of sensitivity of the animals with age may be the result of a change of sensitivity of the reacting mechanism itself with advancing age of the animals.

Previous investigators who have studied toxic placental extracts with regard to toxemia of pregnancy have not reported a desensitization to the toxin.

During the process of inactivation of toxin by serum, *in vitro* the inactivating potency was gradually used up, or itself inactivated in some manner (2). The desensitization of the mice by a preliminary dose of toxin could hardly be explained as the result of similar loss of inactivating potency *in vivo*. Not only is the dose of toxin far too small for a pronounced effect on the total available

inactivating system, but it is to be questioned whether such a change would leave the mice more sensitive to further toxin rather than less sensitive. Further, no significant change of inactivating titre was found experimentally during the refractory period. Hence it is to be suggested that the desensitization following sub-lethal doses involved a change in some portion of the reacting mechanism itself.

Perhaps the increased incidence and type of anatomic lesions following massive doses of toxin during the refractory period are to be interpreted not so much as a measure of quantity of toxin injected but rather as a measure of efficiency of inducing tissue intoxication without inducing the extensive state of collapse leading to immediate death.

The possibility of administering massive doses of toxin during the refractory period may make possible further pathological studies. Not only can focal liver lesions be obtained at will without loss of a large portion of the animals due to prompt death, or wastage of a large and uncertain number of the surviving animals that fail to develop lesions, but kidney lesions become available as well. Similar massive injection during a refractory period in larger animals has been performed and may yield results of interest.

SUMMARY

1. Desensitization to a toxic globulin extractable from placental tissue was studied in mice.

2. The validity of the mouse assay for the toxin by direct proportionality between the minimum lethal volume and the dilution of toxic extract was established experimentally.

3. The toxin was effective only when injected intravenously.

4. Mice became more sensitive, per gram of body weight, as they grew larger and older.

5. Hence, to attain the highest accuracy in the mouse assay requires that mice of a standard weight such as 20 grams should be used.

6. Following sub-lethal doses of toxin, the mice entered a refractory state; i.e., they were temporarily desensitized.

7. The desensitization permitted administration of massive doses, thus permitting a greater efficiency of tissue damage with minimal risk of killing the animals at the time of injection.

8. Focal liver necrosis regularly followed administration of massive doses of toxic extract.

9. Kidney lesions sometimes followed such massive doses of toxin.

10. Occasionally these animals died after an interval suggesting that death occurred secondarily as a result of the lesions.

REFERENCES

- (1) OBATA, I. *J. Immunol.* **4**: 111, 1919.
- (2) SCHNEIDER, C. L. *This Journal* **146**: 140, 1946.
- (3) OLITSKY, P. K. AND J. CASALS. *Proc. Soc. Exper. Biol. and Med.* **60**: 48, 1945.

PLACENTAL TOXIN: INACTIVATION AND TOLERANCE DURING PREGNANCY

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Blood contains an inactivator for a placental toxin (1). The potency, or titre, of blood for this inactivation can be measured (2). In the investigations reported herein, it is seen that the inactivating potency rises during human pregnancy and during pregnancy of mice.

With the mice it was possible, in addition, to study the change of sensitivity to the toxin during pregnancy. Despite the rise of inactivating titre, the pregnant mice became more sensitive to the toxin during pregnancy.

METHOD. Extracts of fresh placenta were used as reagent for the measurement of the inactivator, except in the experiments of figure 1. When needed, the frozen extracts were thawed, centrifuged, assayed and diluted to appropriate concentrations. The toxicity, approximately 200 units per gram of placenta, was well maintained for many days, but not indefinitely; the extracts were discarded when the activity fell below 100 u/gram.

The toxic extracts used as reagent for the experiments of figure 1 were prepared from dry placental powder. This was obtained by drying the thinly spread, perfused placental tissue in the draft from a fan, or by drying from the frozen state.

The mouse blood, 0.10–0.20 cc., was withdrawn from the lateral tail vein, into saline, with which it was diluted four times; the diluted serum was separated from the clot with the aid of centrifugation. Human serum was obtained from clotted blood.

Mixtures of toxic extract of pH 7.4, different amounts of diluted serum, and sufficient saline to yield an initial toxin content of 20 u/cc. were prepared, the serum being added last in each case. The inactivating potency, in units, was that dilution, determined by interpolation, which brought about 50 per cent inactivation of the toxic extract under the previously set forth conditions (2).

The day of pregnancy was determined from the subsequent date of delivery, or was determined approximately from the length of fetuses measured at sacrifice in mice, and from the last menstrual period in humans.

RESULTS. The inactivator contained in serum had the properties of a **protein**. It was inactivated by heating the serum to 85°C. for ten minutes. It did not dialyse through a cellophane membrane. It was precipitated by half saturated ammonium-sulfate solution in the cold, and was poorly soluble in distilled water, but was soluble in physiological sodium chloride solution.

The rise of inactivating potency during human pregnancy is shown in figure 1. The increased titre began after ten weeks of pregnancy, the maximum titre occurring apparently before term; normal levels were restored within a few weeks after delivery.

The maximum titre during pregnancy averaged 230 u/cc., almost six times the normal level. The titre for men averaged 37 u/cc., for non-pregnant women 43 u/cc. of serum.

Figure 2 shows the similar rise of inactivating titre of mouse blood, during mouse pregnancy. The maximum titre apparently occurred before term; it averaged approximately two and one-half times the normal value.

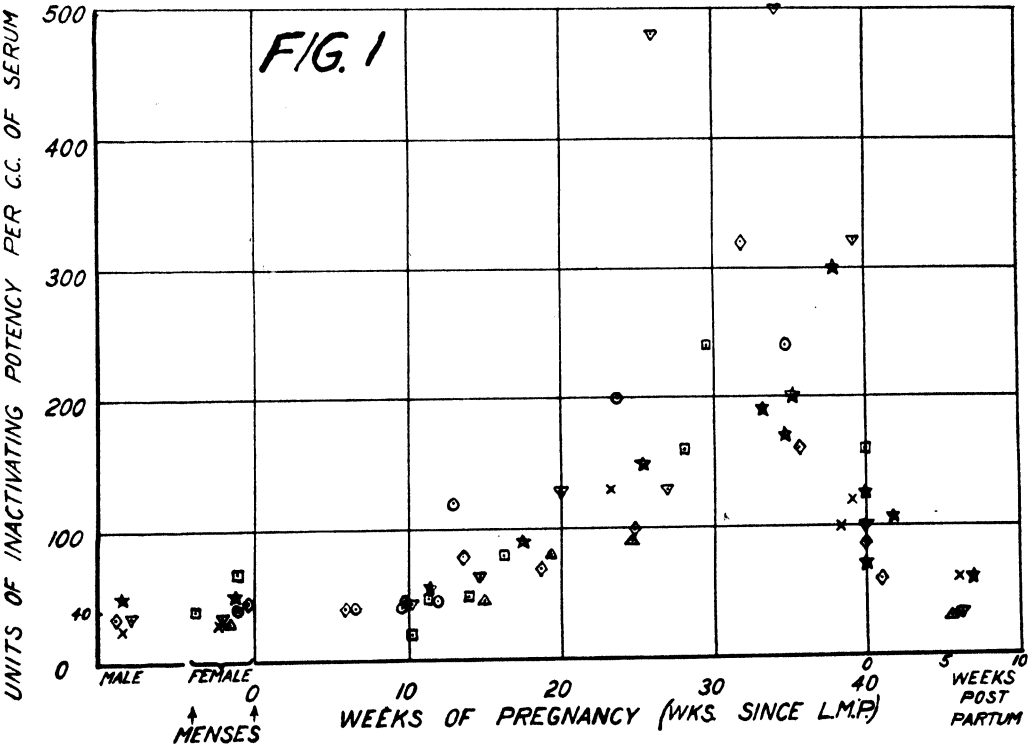


Fig. 1. The detoxifying potency of human serum during pregnancy, measured in weeks since the last menstrual period (L. M. P.).

Fifty points from fifty different patients. Different symbols (crosses, squares, circles, etc.) represent separate experiments, the samples of serum in each experiment being accumulated in the refrigerator over an interval of a few days. Non-pregnant female controls were taken during different stages of the menstrual cycle. Male controls shown for comparison.

In figure 3 the MLV (Minimum Lethal Volume) per 20 grams of body weight was plotted for control mice and for pregnant mice. It is clear that the sensitivity to the extract increased during pregnancy. The ratio of this increase per 20 grams of body weight was almost four to one (3.7 to 1). Since at least part of the increased body weight was represented by fetal bodies, membranes and amniotic fluid (approximately 8-10 grams) and was therefore not immediately permeated by the toxin, if at all, the MLV per (whole) mouse (rather than

per 20 grams of mouse) was also calculated; the increase of sensitivity during pregnancy, per (whole) mouse, was 2.2 to 1.

Whether compared on the basis of MLV, per 20 grams of body weight, or per whole mouse, the MLV for a post-partum mouse did not return to as high a level as for the original non-pregnant mouse. A similar tendency toward higher sensitivity occurs as mice grow older and heavier, although not pregnant. About one-third of the difference for post-partum mice could be accounted for in this manner.

In contrast to non-pregnant mice, only very rarely was liver necrosis or other evidence of tissue damage encountered in pregnant mice following ordinary sub-lethal doses of toxin. The pregnant mice were not easily desensitized. However, with careful repetition and increase of doses, a desensitization or refractory period could be induced. Then massive doses (20–40 u), given during the refractory period, regularly resulted in focal liver necrosis. This was the

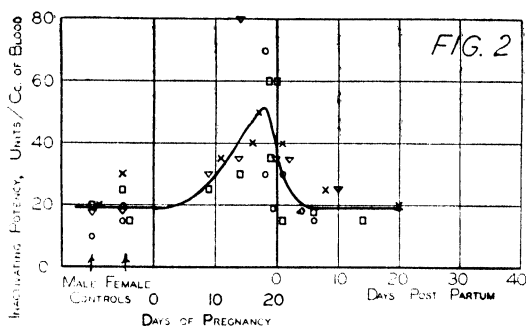


Fig. 2. Inactivating potency in units (titre) per cubic centimeter of blood of mice during pregnancy. The values for blood of male mice and of non-pregnant female mice are shown as controls.

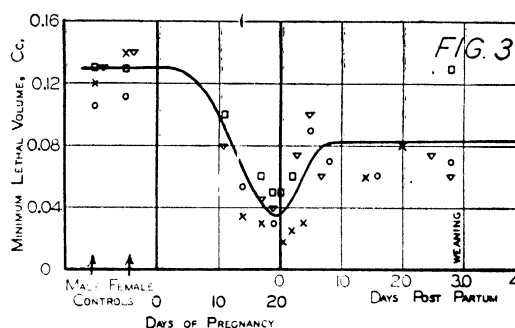


Fig. 3. Minimum lethal volume, per 20 grams of body weight, for pregnant mice. The doses for male mice and for non-pregnant female mice are shown as controls.

same response as obtained from non-pregnant mice after receiving massive doses during the refractory period which could be induced readily by single, just sub-lethal doses of toxin. After treatment with massive doses the pregnant mice, like the non-pregnant ones, besides the focal liver necrosis, occasionally had blood in the small intestine, or in the large mesenteric node, and some of them developed grossly visible, bilateral cortical kidney lesions. The fetuses were commonly found dead at sacrifice if the animals had not already aborted before sacrifice. Whereas death following ordinary sub-lethal doses of toxin rarely occurred, death following massive doses sometimes occurred in the pregnant mice after several hours or a few days.

DISCUSSION. In contrast to the increasing titre of inactivator during pregnancy, reported above, Obata (1) found "that normal human serum, whether from men or from women that are pregnant or not in that condition, possesses a practically uniform power of neutralizing the poisonous property of the pla-

cental extract." A specific reason for the discrepancy between his results and those reported here is not readily available; in view of the difficulty of obtaining and maintaining suitable toxic extracts as reagent for measuring the inactivating system, it is perhaps not surprising that diverse results are reported.

That the inactivating potency of serum for the placental toxin increases during both human pregnancy and pregnancy of mice suggests some common physiological significance for the inactivator.

However, despite the greater inactivating potency of her serum, the pregnant mouse is much more sensitive to the placental toxin than the non-pregnant one.

This raises the question whether the inactivator studied here is the "normal" inactivator of the placental toxin. It is conceivable that the increased inactivating potency might result in tolerance of a greater total amount of toxin, if the toxin were released slowly, rather than being administered rapidly as under the experimental conditions employed, but no special evidence is available for such a contention.

A possible basis on which the increased sensitivity might be a result of the increased level of inactivator is that the initial product or an intermediate product of the reaction between the placental toxin and the inactivator is in some way a step in the response of the animal to the toxin. Such a relationship in the reaction of antigen and antibody in the scheme for desensitization in allergy was suggested by Bronfenbrenner (3). Since the inactivator of placental toxin *in vitro* requires an appreciable time, up to two hours to reach a steady state, increase of the concentration of either factor should increase the rate of formation, and hence the blood level, of the product. In such a scheme an increased level of inactivator *in vivo* should result in a greater sensitivity to rapidly administered doses of toxin.

Although sensitivity of the immediate response to the toxin, namely, the lethal response, was increased during pregnancy, the sensitivity for anatomic lesions did not appear to be greatly altered. Sub-lethal doses of the magnitude that pregnant mice could withstand have been poorly effective also in producing lesions in non-pregnant mice; meanwhile the three or four times larger doses which were just sub-lethal for non-pregnant mice did result in lesions in some of them. Lesions were regularly obtained in both pregnant and non-pregnant mice when massive doses of 20 units or more were given during a period of desensitization. Thus the desensitization of pregnant and of the non-pregnant mice to the immediate (lethal) response to massive doses, rather than also sparing the animal from the development of anatomic lesions could be thought of as permitting time during which the lesions became manifest.

There is a general similarity of the rise of inactivating potency of serum during pregnancy, as studied here, with the rise of histaminase activity of whole blood, as studied by Werle and Effkemann (4, 5). The curves are not identical in detail; e.g., in contrast to the latent interval of ten weeks before appreciable rise of titre found here, the histaminase activity was reported to increase within the first two weeks of pregnancy.

Obata (1) reported a marked decrease of "neutralizing power" of serum of

eclamptic patients during the convulsive attack and Dieckmann (6) reports confirmation of his finding. In the present work, data for titres of blood of eclamptic and pre-eclamptic patients were insufficient for an adequate comparison. In view of the increased sensitivity of mice to placental extracts while at the same time having a high inactivating potency, the question becomes all the more uncertain whether during toxemia of pregnancy, a decreased "neutralizing power" should be interpreted as a cause or as a result of the disease.

By direct proportionality according to weight, the lethal dose for a pregnant woman near term, as compared with a pregnant mouse, should be approximately the amount that can be extracted from four grams of placenta (4 cc. of extract when 1 cc. represents 1 gram of placenta). Even this may be an excessive estimate, for dogs (male) were found to be two and one-half times as sensitive as would be calculated on a weight basis.

SUMMARY

1. The inactivating potency, or titre, of serum for a placental toxin increased to a maximum value at or near the termination of human pregnancy.
2. A similar rise of inactivating titre occurred during pregnancy in mice.
3. Despite the greater inactivating potency of her blood, the pregnant mouse was much more sensitive to the placental toxin than the non-pregnant one.
4. Presumably as a result of this higher initial sensitivity, pregnant mice were more difficult to desensitize to the toxin than were non-pregnant mice.
5. Massive doses of toxin given during the desensitization (refractory period) resulted in extensive focal lesions of the liver and kidneys of pregnant mice just as in non-pregnant mice.

REFERENCES

- (1) OBATA, I. J. Immunol. **4**: 111, 1919.
- (2) SCHNEIDER, C. L. This Journal **146**: 140, 1946.
- (3) BRONFENBRENNER, J. Annals of Allergy **2**: 472, 1944.
- (4) WERLE, E. AND G. EFFKEMANN. Arch. f. Gynakol. **170**: 82, 1940.
- (5) WERLE, E. AND G. EFFKEMANN. Klin. Wchnschr. **19**: 717, 1940.
- (6) DIECKMANN, W. J. The toxemias of pregnancy. 1941.

CHANGES IN ARTERIAL AND VENOUS BLOOD PRESSURE AND FLOW DISTAL TO A CUFF INFLATED ON THE HUMAN ARM

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The effects produced upon the circulation in the limbs by inflating blood pressure cuffs around them have been of considerable interest to both physiologists and clinicians (1, 2, 3, 4). In previous studies (5) we have shown that inflating cuffs placed proximally on the limbs at pressures less than diastolic, reduces the blood flow distally when as a result of the venous obstruction, the local venous pressure rises approximately to cuff pressure. In the present communication we shall report the more immediate effects of inflating such cuffs at pressures lower and higher than diastolic upon the distal arterial and venous pressures as well as upon the blood flow through the extremity.

METHODS. Eleven subjects, after resting quietly in the supine position for twenty minutes, were studied as follows. Brachial arterial and venous pressures were recorded optically by Hamilton manometers (6) attached to needles inserted at the antecubital space. A blood pressure cuff was placed high on the upper arm, well above the needles (upper cuff), another cuff was placed around the forearm, below the needles (middle cuff), and a third about the wrist (lower cuff). The pressure in the upper cuff was recorded by a Hamilton manometer, while that in the middle and lower cuffs was noted in the protocol.

In other studies, plethysmographic blood flows were measured in the forearm or calf of seven subjects by a standard venous occlusion method (7), using collecting pressures in the upper cuff both lower and higher than diastolic. The average of four consecutive or alternate measurements using the same collecting pressure was accepted as the blood flow at that pressure.

RESULTS. *Arterial pressure.* There was no change, either immediate or delayed, in the distal brachial arterial pressure when the upper cuff was inflated at various pressures below diastolic arterial pressure (fig. 1A). When the upper cuff was inflated at moderately supradiastolic pressures, there were no important changes in the distal arterial pressure until the cuff had been inflated for several seconds, and venous pressure had begun to approximate the cuff-pressure (fig. 2). Then the diastolic descent of the arterial pulse waves began to decrease, resulting in an elevation of the recorded "diastolic" arterial pressure. If the cuff was then released, allowing venous pressure to drop, this enhancing effect on diastolic arterial pressure was instantly abolished.

A similar but *immediate* enhancement of diastolic (and mean) arterial pressure could be produced by the same pressure in the upper cuff, if first the distal blood flow were reduced (by inflating the upper cuff to a lower pressure for a moment to congest the veins, or the middle and/or lower cuff to compress or occlude

the circulation in the forearm, or by cooling the body to induce sympathetic vasoconstriction). Moreover, an immediate rise in diastolic pressure often could be produced without first reducing blood flow distally by applying a somewhat higher (but subsystolic) pressure in the upper cuff (fig. 1B). Thus, the immediate increase in the recorded diastolic arterial pressure that occurred upon inflation of the upper cuff was found to be greater the lower the peripheral blood flow and the higher the pressure used in the upper cuff (within limits),

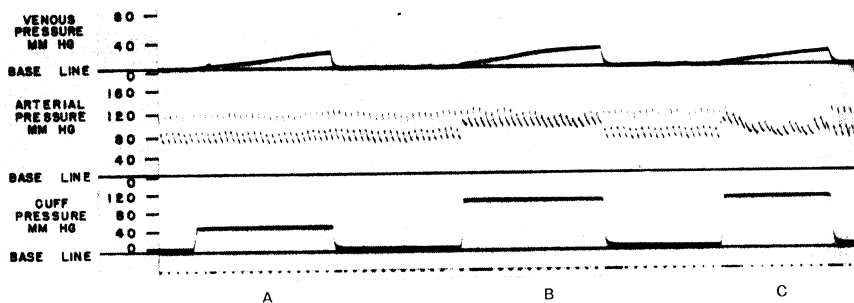


Fig. 1. Optical record of antecubital venous pressure (upper tracing), brachial arterial pressure (middle tracing), and upper cuff pressure (lower tracing) measured with Hamilton manometers in the right arm. The signal marker below registers intervals of one second (short marks) and also the time of inflation and deflation of the upper cuff (long marks). Scales appended at the left are in millimeters of mercury. Prior to the time of the record, the circulation in the right hand had been occluded by a cuff around the wrist inflated at 200 mm. Hg.

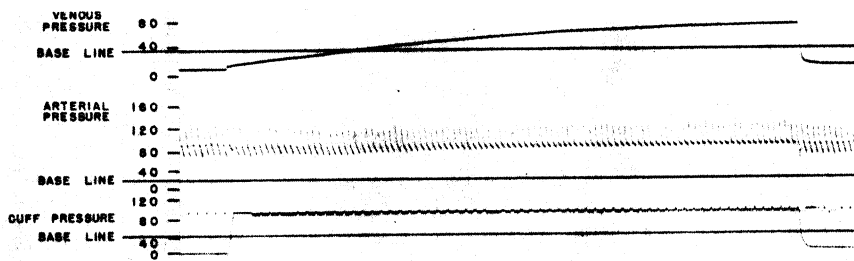


Fig. 2. Optical record of antecubital venous pressure, brachial arterial pressure and upper cuff pressure. Other notations as in figure 1.

However, a point was always reached above which any further increment of pressure in the upper cuff would result in a fall of systolic arterial pressure, although diastolic (and mean) pressure might rise as described above (fig. 1C), or might also fall, depending upon the state of the vascular bed distally. Thus, if there were vasodilatation and little congestion distally, the diastolic (and mean) pressure would be more apt to fall when higher pressures were applied, whereas if there were vasoconstriction (or compression) and congestion distally, the diastolic (and mean) pressure would be more apt to rise. Associated with the immediate changes in mean arterial pressure were corresponding changes in the initial rate of rise in the venous pressure, which rose faster the higher the

pressure in the artery distal to the cuff (fig. 1). Also, as venous pressure rose to high values and leveled off, the systolic arterial pressure, if lowered, returned toward the control value, while diastolic pressure increased toward cuff pressure (fig. 1C, fig. 2).

The effects on the distal arterial pressure when the upper cuff was inflated quickly to pressures greater than systolic arterial pressure were as follows (figs. 3 and 4). The arterial record was first interrupted by a sharp rise in pressure, presumably due to the mechanical compression of the artery by the

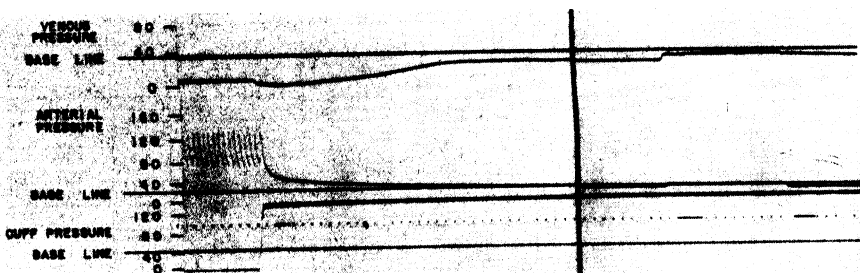


Fig. 3. Optical record of antecubital venous pressure, brachial arterial pressure, and upper cuff pressure. At the black vertical line a section of the record of thirty seconds' duration is omitted. At the first long signal after the omission, a pressure of 30 mm. Hg. was applied in a cuff around the right forearm; at the second long signal it was released. Other notations as in figure 1.

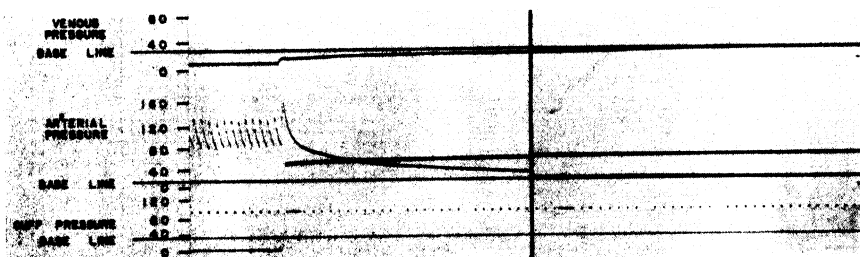


Fig. 4. Optical record of antecubital venous pressure, brachial arterial pressure, and upper cuff pressure. At the vertical black line a section of the record of thirty seconds' duration is omitted. At the long signal mark after the omission, a block of ice with surface of approximately 100 sq. cm. was applied briefly to the skin of the chest. Other notations as in figure 1.

cuff. The height to which this spike carried the pressure depended upon the level at the instant of occlusion, being higher when superimposed on a systolic crest (fig. 4) than on a diastolic trough (fig. 3). The pressure next fell off rapidly in a smooth curve at a rate and to a depth that seemed to vary inversely with the resistance in the vascular bed distal to the occluding cuff and the volume of blood filling it. Thus, the pressure fell off quicker and to lower levels if the middle cuff had previously been inflated for some minutes and was released so that at the moment of the occlusion by the upper cuff the distal bed was empty and in a state of reactive vasodilatation. Contrariwise, the pressure fell

more slowly when either the middle cuff was left inflated after the occlusion, or the veins had previously been congested by a subsystolic pressure in the upper cuff. When conditions distally were kept constant, the pressure fell to the same level eventually, but required longer when the initial spike of pressure was higher. Arterial pressure always fell as low as recorded venous pressure and in special instances, as described later, was found to fall below it.

Venous pressure. In some experiments when the upper cuff was too close to the antecubital needle or rode down, there was a sudden sharp rise in the recorded venous pressure upon inflation of the cuff. This was presumed to be due to mechanical compression of the blood in the isolated venous segment between the cuff and the venous valves just distal to the manometer needle. In other experiments there was only a slight initial mechanical effect, followed by a smooth, steady rise in venous pressure to approximately cuff pressure, and then a leveling off (fig. 2). When the venous pressure reached higher levels, pulsations appeared in the tracing synchronous with those in the arterial record.

Since the venous segment in which pressure was being recorded was probably in free communication with the distal venous system during the steady rise of venous pressure after inflation of the upper cuff, rising pressures were assumed to reflect the changes in distal venous pressure. The rate of rise in venous pressure was found to be uniform at different pressures in the upper cuff up to diastolic and somewhat above. However, when the cuff was inflated at pressures higher than diastolic, there was often a slightly *faster* rise in venous pressure. This was found to occur only when the distal mean arterial pressure was also increased, as already described (fig. 1B). However, a point was reached above which further increases of the cuff pressure caused a decrease in the rate of rise of venous pressure (and a decrease in the distal mean arterial pressure) (fig. 1C).

When the upper cuff was quickly inflated to a pressure greater than systolic, there was again an initial mechanical effect upon venous pressure, which when slight was followed for some seconds by a gradual smooth rise and then a leveling off (fig. 3). It was assumed that during the gradual rise of venous pressure the vein segment again was in free communication with the venous system distally. As venous pressure rose, arterial pressure fell to approximate it, but venous pressure reached its maximum before arterial pressure reached its minimum. Therefore, it could not be judged exactly when the venous segment was still in free communication, or when it was isolated by closure of venous valves lower down. That it probably became so isolated was shown when arterial pressure actually fell to levels lower than the recorded venous pressure. This occurred when resistance in the vascular bed of the forearm had been lowered by preliminary inflation of the middle cuff for some minutes (to express blood and induce reactive vasodilatation), followed by deflation at the instant of applying the occluding pressure in the upper cuff, or after long periods of occlusion by the upper cuff without preliminary preparation. After a period of circulatory occlusion during which arterial pressure had fallen to approximately venous pressure, it could be made to fall below venous pressure if the muscles of the

forearm were first briefly compressed or contracted (by inflating the middle cuff (fig. 3), or by asking the subject momentarily to flex his fingers). During the compression or contraction, the arterial and venous pressures rose sharply, and after the release they fell, but the arterial pressure fell more and to lower levels than the venous (fig. 3). A more delayed, slower and smoother rise in both pressures could be produced when they had become stabilized after a period

TABLE 1

Plethysmographic blood flow measured in the limbs using an infradiastolic (control) pressure in the upper collecting cuff as compared with the supradiastolic pressure found by trial and error to produce the highest (optimum) flow

Each measurement given is the average of four alternate flows done with the upper cuff inflated at the pressure noted.

SUBJECT	DIAGNOSIS	ARTE- RIAL PRES- SURE	LIMB AND VOLUME	CONTROL UPPER CUFF PRESSURE AND FLOW	OPTIMUM UPPER CUFF PRESSURE AND FLOW	UPPER CUFF PRESSURE CAUS- ING DECREASE IN FLOW
1	Chronic osteo- myelitis	123/75	Arm 830 cc.	55 mm. 40 cc./min.	100 mm. 53 cc./min.	Above 110 mm.
2	Hysteria ? epilepsy	100/65	Arm 540 cc.	50 mm. 19.5 cc./min.	85 mm. 24 cc./min.	Above 92 mm.
3	Diabetes mellitus	146/72	Arm 665 cc.	70 mm. 20.7 cc./min.	115 mm. 22.7 cc./min.	Above 140 mm.
4	Syphilitic aortic regurgitation	190/60	Arm 590 cc.	50 mm. 21.1 cc./min.	168 mm. 29.4 cc./min.	Above 180 mm.
4	Syphilitic aortic regurgitation	190/60	Leg 990 cc.	50 mm. 30.5 cc./min.	168 mm. 41.0 cc./min.	Above 180 mm.
5	Peptic ulcer	126/82	Arm 720 cc.	55 mm. 23.3 cc./min.	102 mm. 26.5 cc./min.	Above 110 mm.
5	Peptic ulcer	126/82	Leg 1,030 cc.	55 mm. 25.2 cc./min.	102 mm. 28.0 cc./min.	Above 110 mm.
6	Asthma	125/74	Arm 780 cc.	65 mm. 21.4 cc./min.	100 mm. 22.6 cc./min.	Above 100 mm.
7	Psychoneurosis	134/70	Arm 900 cc.	65 mm. 27.3 cc./min.	120 mm. 33.3 cc./min.	Above 120 mm.

of circulatory occlusion, if a vasoconstrictor stimulus, such as a block of ice, were applied to a remote part of the body (fig. 4). This response appeared about three seconds after the stimulus, continued with a slight progressive rise in both arterial and venous pressure for about seven seconds, and then slowly subsided, as indicated by a gradual fall in arterial pressure below venous pressure, which showed little, if any, further change.

Blood flow. During venous occlusion plethysmography, it was observed that the blood flow, as indicated by the rate of increase in volume of the arm or leg after inflation of the upper cuff, was uniform under controlled conditions when various pressures up to and slightly greater than diastolic arterial pressure were used in the cuff. Confirming previous experience, until now not understood, it was found that pressures in the cuff moderately greater than diastolic instead of impeding the blood flow often enhanced it. By trial and error it was usually possible to find a supradiastolic pressure that produced a rate of flow consistently higher than that observed in alternate control measurements using any infradiastolic pressure in the upper cuff (table 1, fig. 5). A pressure that caused an increased flow could be found more readily when the subject had a high arterial pulse pressure and/or a low control blood flow. The increased flow was asso-

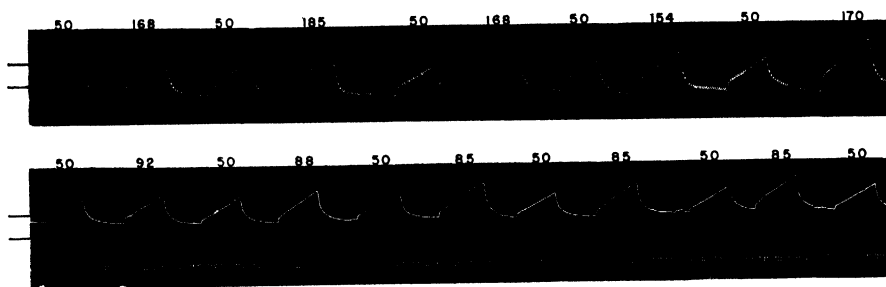


Fig. 5. Plethysmographic record of blood flow in the forearm of a subject with arterio-sclerotic and luetic disease of the aorta and an arterial pressure of 190/60 mm. Hg (upper tracing), and of a normal subject with an arterial pressure of 100/55 mm. Hg (lower tracing). The collecting pressure applied in the upper cuff for each measurement of blood flow is given in millimeters of mercury just above the respective flow. The scale appended at the left indicates a volume of 5 cc. The time marker at the bottom indicates seconds. The steepness of the blood flow curves indicates the rate of blood flow. Note that alternate control measurements with an infradiastolic collecting pressure (50 mm. Hg) are less steep than measurements with supradiastolic collecting pressures (within limits), and that the pulsations are greater. When too high a collecting pressure was applied (fourth flow, upper tracing), the measurement was less steep than the controls.

ciated with a decrease in the size of the recorded pulsations. The optimal level of pressure in the cuff to produce the maximal flow distally in the limb varied markedly in different subjects and could not be predicted with any accuracy. If this level of pressure was exceeded by even a few millimeters of mercury a marked decrease in blood flow often occurred.

Thus, the experience with the venous occlusion plethysmograph confirmed the impression derived from observing the rise in venous pressure when the upper cuff was inflated at different pressures, namely, that the rise was more rapid when such a supradiastolic pressure was used in the upper cuff as to cause a rise in the distally recorded mean arterial pressure.

DISCUSSION. The immediate increase in "diastolic" (and mean) arterial pressure produced distally by inflating at suitable pressures a cuff placed high on a limb was the most remarkable phenomenon observed in this study. It may

be explained as follows. The cuff served as an adjustable valve upon the artery, compressing it and isolating the distal arterial tree from the central system whenever pressure in the cuff exceeded that within the artery. Thus, when inflated at a pressure less than diastolic, the cuff did not isolate the distal arterial tree from the central *Windkessel* nor disturb the usual hemodynamic factors determining the arterial pressure recorded distally. At a pressure greater than systolic, the cuff did isolate the distal tree and abolish the effects of central hemodynamic factors upon the arterial pressure recorded distally. Inflated at a pressure between diastolic and systolic, the cuff isolated the distal tree only intermittently, i.e., during those portions of the pulse cycles when pressure in the cuff exceeded pressure in the artery under the cuff. During these intermittent periods of isolation, the cuff again abolished the effects of central hemodynamic factors upon the arterial pressure recorded distally. One of the chief effects abolished was that due to the sizable reflux of blood that has been shown to occur in many arteries during the diastolic period (8, 9). Thus, the cuff acted as a valve to trap, for the remainder of the cycle, an increased volume of blood in the distal arterial tree, causing an increased "diastolic" pressure.

Such an enhancement of "diastolic" pressure distal to a cuff inflated at a pressure between systemic diastolic and systolic pressure was considered to be dependent upon two factors: 1, the net amount of blood added during the periods of patency of the artery under the cuff to the volume of blood within the distal arterial tree; 2, the amount of resistance in the distal arterial tree during the periods of isolation. 1. The net amount of blood added to the distal arterial tree during patency would equal the amount injected under the cuff in the systolic phase minus the amount regurgitated in the diastolic phase. Both of these amounts, in turn, would be influenced by the duration of patency, which would be governed by the relationship between the pressure in the cuff and that in the artery under the cuff. Obviously the higher the pressure in the cuff, the shorter the period of patency and the smaller the amounts injected and regurgitated. But the amount regurgitated would also depend upon the resistance to flow distally. Thus in a given time, little regurgitation would occur with a low peripheral resistance, and more with a high peripheral resistance. Having these considerations in mind it follows that with a given central arterial pressure and a fixed peripheral resistance the pressure attained distally at the end of the period of patency would depend solely on the pressure applied in the upper cuff.

2. With a given pressure in the distal arterial tree at the end of the periods of patency, the changes in pressure that would occur subsequently during the periods of isolation would depend on the peripheral resistance to outflow from the distal tree. Thus, if the resistance to outflow were great, the diastolic fall in pressure distally would be less than occurred simultaneously in the central *Windkessel*, enhancing the "diastolic" pressure. If, on the other hand, the resistance were low, the diastolic fall in pressure in the isolated tree might be greater than occurred centrally, immediately lowering "diastolic" pressure. In the latter instance, furthermore, the injection of blood during the next period of patency of the artery under the cuff would encounter a relatively emptier

distal arterial tree and therefore would fail to raise the "systolic" pressure as high as that found centrally. As already explained, distal systolic pressure would also be lowered whenever the net injection of blood during patency was too small (from too high a pressure in the cuff and too brief a period of patency) although, depending upon the resistance to outflow distally, the "diastolic" pressure might be raised, unchanged or lowered. Examples of all these theoretical possibilities were encountered in the experiments.

With a relatively stable peripheral resistance, blood flow in the area distal to the inflated cuff (as indicated by the rate of rise of venous pressure or of limb volume) varied with the changes in mean arterial pressure distal to the cuff. When the mean arterial pressure was enhanced distally the blood flow would increase, but only until venous pressure had risen high enough to impede the flow. When mean arterial pressure was lowered distally the blood flow would decrease as compared with control values, and theoretically would continue to decrease until it just equalled the input of blood during patency of the artery under the cuff. Actually because venous pressure rose, increasing the resistance, the flow decreased still further, and the distal arterial pressure rose, first in its "systolic" and then in its "diastolic" components. Thus, in the distal arterial tree, as in systemic hemodynamics, pressure and flow are inseparably related to each other and to the volume of blood and the resistance in the vascular compartment concerned.

As stated above, the increase in peripheral blood flow during an enhancement of distal arterial pressure would be maintained only until venous pressure had risen high enough to impede the flow (4, 5). In normal subjects the time of the increase, theoretically, would vary from about 15 to 60 seconds after inflation of the cuff, depending upon the control rate of flow and the capacity of the venous bed freely to distend. Hence, the enhancing effect could be obtained only intermittently and at unpredictable intervals. Likewise, the optimal cuff pressure to produce the maximal effect could not be judged unless either distal arterial pressure or blood flow were being recorded. If this optimal pressure were exceeded by even a few millimeters of mercury a detrimental effect might be produced. Finally, confirming earlier experience, it was found that the increases in blood flow produced by higher arterial pressures in the limbs were relatively small as compared with those that occur after a vasodilatation. These considerations made the practical usefulness of the method as a therapeutic procedure appear to be doubtful. Nevertheless, it cannot be denied that it would be theoretically most effective as a means of increasing blood flow in just those patients who might need it most, namely, those with a minimal peripheral flow and a high pulse pressure due to arteriosclerosis. Hence, it could not be excluded as possibly of therapeutic value, and indeed conceivably might have produced the beneficial effects reported to have followed the use of intermittent inflation of cuffs on the limbs (2, 3). Our own limited experience with it, however, has been discouraging. Not only have the clinical results been poor, but one is constantly in fear of doing damage by impeding instead of enhancing flow or by traumatizing devitalized tissues under the cuff.

The fact that after complete circulatory occlusion the arterial pressure fell at least to venous pressure and did not come to rest at any point higher than that to be expected in the capillary-venous system, indicates that the artery was at all times in free communication with the capillaries through the arterioles and did not maintain a pressure independently. The results of these experiments corroborate the work of others that shows that after arterial occlusion, forward capillary flow continues for some length of time, up to about a minute, and presumably ceases only when the pressure in the arterial tree is lowered to that in the post-arteriolar beds. Flow may then actually become reversed, indicating that arterial pressure has fallen below capillary pressure (10). It is of interest that during such an occlusion evidence of active vasoconstriction may be elicited by using a common vasoconstrictor stimulus. The timing and nature of the rise in arterial and venous pressures after the stimulus strongly suggests that it represented a sympathetic vasoconstrictor response in the distal vascular segment.

SUMMARY

The effects of inflating a cuff around the upper arm upon arterial and venous pressure distally were recorded with Hamilton manometers. When inflated at pressures less than diastolic, the cuff caused no change in distal arterial pressure, but produced a prompt, steady rise in venous pressure to about the same level as that in the cuff. When inflated at pressures greater than diastolic, the cuff caused either no change, an increase, or a decrease in arterial pressure distally, depending upon the pressure in the cuff and the resting blood flow in the part. Thus, the higher the cuff pressure (within limits) and the lower the flow, the greater the enhancement of diastolic and mean arterial pressure distally. When pressure in the cuff was too high it caused a decrease in systolic and mean pressure.

Venous pressure rose when the upper cuff was inflated at supradiastolic pressures similarly as at infradiastolic pressures, except that the rate of rise varied directly with the resultant distal mean arterial pressure. Likewise, the rate of rise of plethysmographic blood flow measurements could be increased by applying the proper pressure in the upper cuff. These findings indicate that increases in distal arterial pressure produced by inflating the cuff at certain supradiastolic pressures are due to a reduction of regurgitant arterial flow and are associated with increases in forward blood flow distally. Such increases are maintained only briefly until venous pressure and resistance rise high enough to impede the flow.

When the cuff was inflated at suprasystolic pressures, distal arterial pressure fell in a smooth curve to, or slightly below, venous pressure, which rose.

The theoretical mechanisms and possible practical applications of these phenomena are discussed.

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REFERENCES

- (1) LEWIS, T. AND R. GRANT. Heart **12**: 73, 1925.
- (2) COLLINS, W. S. AND N. D. WILENSKY. J. A. M. A. **109**: 2125, 1937.
- (3) LINTON, R. L., P. J. MORRISON, H. ULFELDER AND A. L. LIBBY. Am. Heart J. **21**: 721, 1941.
- (4) PRITCHARD, W. H., A. S. WEISBERGER, E. F. SCHROEDER, R. E. SHIPLEY AND D. E. GREGG. Fed. Proc. **1**: 68, 1942.
- (5) FRIEDLAND, C. K., J. S. HUNT AND R. W. WILKINS. Am. Heart J. **25**: 631, 1943.
- (6) HAMILTON, W. F., G. BREWER AND I. BROTMAN. This Journal **107**: 427, 1934.
- (7) WILKINS, R. W. AND L. W. EICHNA. Bull. Johns Hopkins Hosp. **68**: 425, 1941.
- (8) GREGG, D. E., W. H. PRITCHARD, R. W. ECKSTEIN, R. E. SHIPLEY, A. ROTTA, J. DINGLE, T. W. STEEGE AND J. T. WEARN. This Journal **136**: 250, 1942.
- (9) SHIPLEY, R. E., D. E. GREGG AND E. F. SCHROEDER. This Journal **138**: 718, 1943.
- (10) MULINOS, M. AND I. SHULMAN. This Journal **125**: 310, 1939.

STUDIES OF RIGHT AND LEFT VENTRICULAR ACTIVITY DURING HEMORRHAGIC HYPOTENSION AND SHOCK¹

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Extensive investigations into the phenomenon of hemorrhagic shock carried out in this laboratory have suggested that peripheral circulatory factors are not the only ones involved in the production of a state of irreversible circulatory failure.

In a recent paper one of us reviewed the evidence that myocardial depression is at least partly responsible for the failure of transfusions to maintain arterial pressure and an adequate circulation in the shock state (1). Such a myocardial depression is indicated by a slowing of the heart and a reduction in cardiac output at comparable venous pressures. The fact that a prolonged period of extreme hypotension is necessary to produce conditions favorable to the development of hemorrhagic shock offers an opportunity for myocardial damage by virtue of reduced aortic pressure and reduced coronary flow. Under such conditions it has been suggested that since the work of the right heart is decreased less than that of the left heart, there is a possibility of primary right-sided myocardial failure. This was a hypothesis and remained to be demonstrated. Since the reduced response to comparable venous pressures can be reasonably expected to be due to either a depression of the contractile function or to changes in cardiac tonus, further study of this point is indicated.

This report deals with two series of experiments in which an effort was made to compare the activities of the right and left ventricles during standardized hemorrhagic shock developed in this laboratory and repeatedly described (2). In brief, the procedure consists of maintaining dogs successively at mean arterial pressures of 50 mm. Hg for 90 minutes and at 30 mm. Hg for another 45 minutes, after which all withdrawn heparinized blood is reinfused. Progressive circulatory failure (shock) develops soon after reinfusion in a large and constant percentage of cases. Autopsies continue to reveal various degrees of congestion, edema, and hemorrhage in the duodenal mucosa, the severity of which is not, however, always related to the speed with which circulatory failure develops.

In both series of experiments dogs were given a preliminary dose of morphine sulfate (ca 3 mgm./kilo) and sodium barbital (ca 200 mgm./kilo). Experimental observations were started about 2 hours after induction of the barbital anesthesia and were continued for 6 to 10 hours in various experiments. No further anesthetic was required during the course of these experiments, an important consideration in studies of cardiac behavior. Rectal temperature was maintained within 1 to 2 degrees of control temperature at the start of the experiment by application or withdrawal of external heat.

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First series. The activity of the left ventricle was inferred from changes in aortic pressure curves and that of the right ventricle from right ventricular pressure curves of naturally breathing dogs. Pressures were recorded by optical manometers of the Gregg type, the former by sounds inserted via a carotid artery and the latter by a sound introduced into the right ventricle via a jugular vein. Information regarding the left ventricle was limited to studies of changes in duration of the isometric and ejection phases of systole. Owing to the fact that the lax arterial system is thrown into violent vibration after bleeding, the aortic pressure pulses do not represent an accurate picture of the ventricular pressure summits. Right ventricular curves were analyzed with regard to changes in contour, initial tension, and duration of phasic changes with respect to cycle length.

In this connection it seems important to emphasize again that use of acceptable optical manometers, with techniques insuring adequate frequencies and carefully repeated calibrations is not itself a guarantee that the recorded curves picture the pressure pulses in blood vessels or chambers of the heart with accuracy. Faithful reproduction of pressure variations is realized only when the openings of catheters or sounds remain unobstructed throughout the cardiac cycle. This is not always easy to realize when a sound is inserted into the right ventricle via the tricuspid orifice, because the trabeculae and papillary muscles easily obstruct the cannula during systole and isometric relaxation when the ventricular cavity is reduced to a very small capacity. Such obstructions produce notching or splintering of the rising limb and superimposed waves on the summit of curves, while sudden release during relaxation causes deformations of the isometric relaxation gradient and even negative suction effects that have misled investigators. Since changes of intrathoracic pressure affect the curves during natural respiration only curves during expiratory pauses were compared. Even so, readings of initial tension do not necessarily reflect changes in effective venous pressures because expiratory intrathoracic pressure varied during the course of our experiments.

Since we (3) and others (4) have questioned whether intrathoracic pressure as commonly measured represents true variations of negative pressure about the heart and large vessels, efforts to study effective initial tensions were abandoned. Nevertheless, comparisons of actual initial pressures during expiratory pauses prove of some interest in relating results to human studies.

Changes in the configuration of right ventricular pressure curves found during hemorrhagic hypotension, after reinfusion, and during spontaneous circulatory failure are shown in three series of transcribed curves of figure 1. In most of these curves, cycle length varied but little, since the heart was accelerated at the start. The curves of series A during hemorrhagic hypotension show a more gradual rise, an earlier summit, and less depression of the pressure summit than occurs in the left ventricle, as judged from systolic pressures. The whole curves are of shorter duration, due chiefly to abbreviation of systolic ejection. During extreme arterial hypotension (30 mm. Hg) the pressure curves have a simple sine form. Initial tension is at first reduced, but increases during the latter part of the

50 mm. Hg period, and still more during the 30 mm. Hg period of hypotension. Reinfusion of blood more than restores the normal amplitude, increases the slope and duration of the curves as shown in series B. Such normal curves are maintained only as long as the initial tension remains definitely above the control of series A. As arterial pressures commence to decline about 15 minutes after transfusion, right ventricular initial tension returns to normal ranges (cf. fig. 1-A, curve 1—fig. 1-C, curves 9 and 10), and the pressure curves come to resemble those following hemorrhage. As circulatory failure progresses initial tension rises, but the form of the right ventricular pressure curves become more rounded and narrow (cf. fig. 1-C, curves 11 and 12.). In other words, despite an increase in initial tension the right ventricular action becomes feebler and duration of systole shorter. If, as seems probable from aortic pressure curves, the left ventricle also reduces its period of contraction at equivalent cycle lengths this

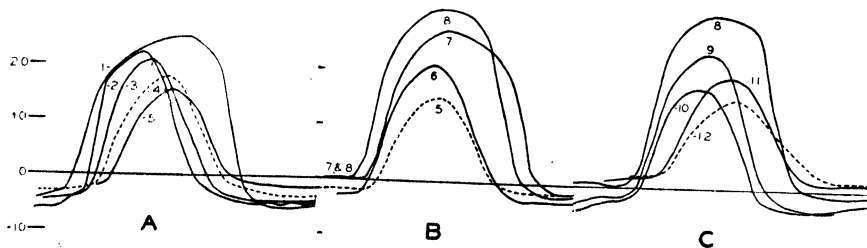


Fig. 1. Transcribed right ventricular pressure curves as follows: A. During hemorrhage and hypotension periods. 1, control A.P. = 125 mm. Hg; 2, 60 minutes after bleeding 54 cc./kilo, A.P. = 60 mm. Hg; 3, 88 minutes after bleeding, A.P. = 50 mm. Hg; 4, immediately after additional hemorrhage 2 cc./kilo, B.P. 30 mm. Hg; 5, after 45 minutes, 30 mm. Hg hypotension. Note fall of initial tension in curves 2-3, slight rise in curves 4 and 5. B. During reinfusion, 5 as above; 6, after reinfusion 19 cc./kilo blood, A.P. = 78 mm. Hg; 7, after infusion 45 cc./kilo, A.P. = 103 mm. Hg; 8, after reinfusion 54 cc./kilo, A.P. = 93 mm. Hg. C. After reinfusion, 8, as above; 9, 46 min. later, A.P. = 100 mm. Hg; 10, 2 hrs. after infusion, A.P. = 60 mm. Hg; 11, 3 hrs. after infusion, A.P. = 70 mm. Hg; 12, 4 hrs. after infusion, A.P. = 30 mm. Hg. Note rise of initial tension in curves 11 and 12. (Successive curves shifted slightly to right for clarity.)

may have some importance in explaining the peaking of aortic pressure pulses; the ejection consists in a rapid shot of blood into the aorta which is not followed, as in normal ejection, by a sustained diminishing flow. It is conceivable that the latter may serve a dynamic purpose in smoothing pressure curves in the aorta.

The abridgement of systole and systolic ejection also occurs in aortic pressure curves after hemorrhage and during post-infusion failure of the circulation. The abridgement of systole calculated in many experiments is greater than predicted from calculated s/c ratios at accelerated heart rates and suggests either excessive sympathetic action or myocardial depression. Both seem to have such an effect on the time course of ventricular contraction (5). In a number of experiments in which the heart rate was initially rapid, no further acceleration, or at times a deceleration, occurred. In such cases, an example of which is plotted in figure 2, both isometric contraction and systolic ejection phases are still reduced when

previous diastole is unchanged or even lengthened (cf. bars 1, 4, 5, 8, 9). These phases are only temporarily restored to control values after reinfusion (cf. bars 10, 11). Thus 10 minutes after transfusion, while arterial pressure was still high, and the cycles were longer, abridgement had begun to set in (cf. bars 1-2 and 12-13). It continued progressively even during very slow terminal beats. Such

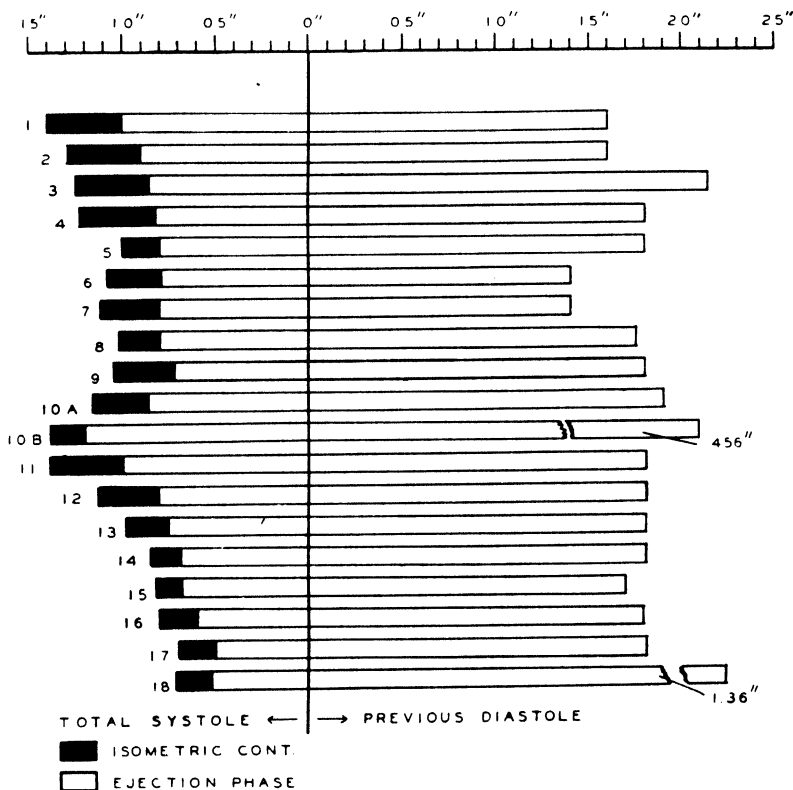


Fig. 2. Plots showing lengths of ventricular phases (isometric contraction, ejection, total systole and total previous diastole. 1, control, A.P. = 122 mm. Hg; 2, 200 cc. hemorrhage, A.P. = 80 mm. Hg; 3, 40 cc. hemorrhage, A.P. = 55 mm. Hg; 4-9 inclusive, A.P. = 44, 62, 52, 34, 38, and 34 mm. Hg respectively; 10A, during reinfusion, A.P. = 74 mm. Hg; 10B, post-compensatory beat; 11, end of reinfusion, A.P. = 118 mm. Hg; 12-17 inclusive, 10, 28, 49, 59, 79, and 82 minutes post-reinfusion, A.P. = 105, 90, 65, 52, 38, and 32 mm. Hg respectively; 18, terminal beat. Time scale in hundredths of a second.

abbreviation of contraction independent of cycle length strongly suggests myocardial failure.

Second series. This series on exposed hearts was undertaken to study more accurately the relation of initial tension, unaffected by variable intrathoracic pressures, on the dynamics of the right and left ventricles during standard hemorrhagic hypotension and shock. The chests were opened wide by midline thoracotomy under artificial respiration, giving meticulous attention to minimize

trauma and loss of blood. The pericardia were split and sewn to the edges of the thoracic walls, thus forming a pericardial cradle. The heart was protected by moist cotton pads and suspended in such a fashion that the inflation and deflation of the lungs did not appreciably disturb the position of the heart. The heart was not elevated sufficiently in the cradle so as to embarrass blood flow in the entering veins, a point checked by careful observation of the veins and arterial pressures. Every precaution was taken to insure constant hydrostatic levels of ventricles.

The right and left ventricles were pierced through the ventricular walls by 15 gauge blunt hypodermic needles. Since the myocardial openings tended to enlarge as a result of continued movements of the heart during prolonged experiments the needles were fitted with small conical sleeves which could be pushed into the heart wall gently and thus effectively control oozing. The ventricular pressures were recorded optically by Gregg type manometers of adequate frequency and such a sensitivity that pressure changes of 1 mm. Hg could be measured easily. The zero level of pressure was referred to the level of the animal board since this was constant. Clotting of blood in the cannulae was prevented by frequent flushes with small quantities of saline. If there was any doubt as to the patency of the cannulae they were easily removed, cleaned, and reinserted. Each optical record was followed by a calibration in relation to some known pressure (0 or 10 mm. Hg) in order to avoid errors due to shifts in the position of the recording beams in relation to their base lines.

Adequate and constant artificial respiration was maintained throughout the experiments with frequent withdrawal and reinjection of small quantities of femoral arterial blood in order to check the color, and thus the adequacy of ventilation. In all probability, the aeration of the blood in these experiments was better than in some shock experiments on closed-chest dogs whose respiration is depressed. Therefore, we feel confident that anoxic anoxia is not a factor to be reckoned with in these experiments. Pressure curves were recorded during a 5 to 10 second cessation of respiration in order to avoid artefacts due to lung inflation. This period of respiratory arrest was not sufficient to produce anoxia or hypercapnia.

The success of these experiments was due in no small part to meticulous care in the operative procedures with special attention to hemostasis. The excellent condition of the animals is indicated by the fact that control mean blood pressures were normal for barbitalized dogs and the bleeding volumes (to 50 mm. Hg) compared favorably with those of intact dogs (ca 4 per cent of body weight). This represents an improvement over results previously realized in this laboratory (6).

The results of these experiments are based on experiments on 10 dogs, five of which terminated in hemorrhagic shock, the others dying in the 50 or 30 mm. hypotensive period or by accidental ventricular fibrillation.

RESULTS. The general changes in form of ventricular pressure curves were similar to those already described. A series of curves showing the improvement following transfusion and changes during subsequent circulatory failure are shown

in figure 3. The quantitative data are presented in the following manner: The experiments can be divided into five phases: control period, 50 mm. Hg. hypotensive period, 30 mm. Hg hypotensive period, reinfusion and post-infusion circulatory failure. These phases are rather constant in time for each experiment with the exception of the post-infusion period preceding circulatory failure. By equalizing the latter, it was possible to plot each of the experiments and superimpose the plots in order to gain some idea of directional changes in right and left ventricular systolic pressures (peak pressures) and initial tensions in each phase of the experiments: From the superimposed plots a set of curves were drawn which, in our judgment, best represented the trend of events in those dogs which developed progressive circulatory failure (solid line, fig. 4). The experi-

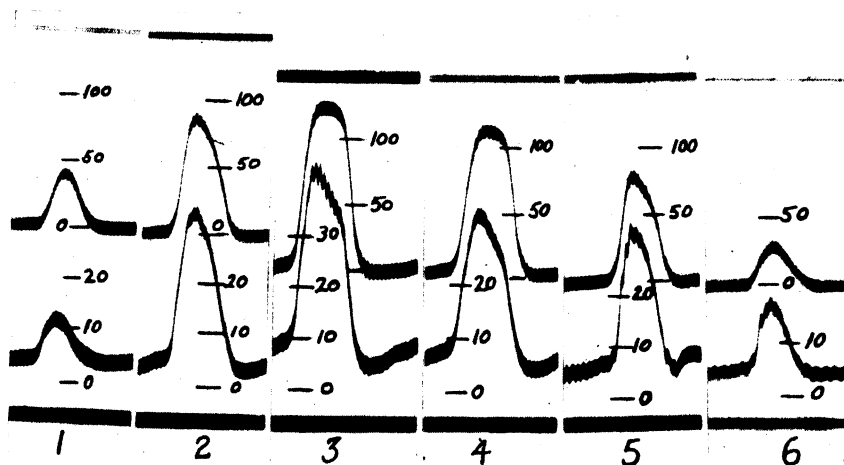


Fig. 3. Series of left (upper) and right (lower) ventricular pressure curves taken as follows: (1) at end of 30 mm. Hg hypotension, (2 and 3) during reinfusion of blood, and (3, 4, 5, 6) during subsequent circulatory failure.

ment (no. 86) which best fitted the "trend curves" was added as a dotted line (fig. 4) and chosen for analysis.

Briefly, the following observations can be made from inspection of the two sets of curves.

Control. The average left ventricular systolic pressure was 120 mm. and the left ventricular initial tension was equal to 9.5 mm. Hg. The corresponding control values for the right heart are 25 and 7.4 mm. respectively. The initial tension of the right ventricle is apparently less than that of the left when the heart is exposed to atmospheric pressure. This was true in all experiments. The control values of experiment 86 coincided with these figures with the exception of the left ventricular pressure which was somewhat higher than the average, but still within normal ranges.

50 mm. Hg period. All pressures fall initially as a result of a rapid hemorrhage. The initial tension of the right ventricle falls somewhat less than that of the left

ventricle, but both tend to return toward control levels near the end of the 50 mm. period. Both the right and left ventricular systolic pressures tend to improve during the 50 mm. period. This was particularly true in experiment 86.

30 mm. Hg period. A further decrease in mean arterial pressure results in a marked lowering of left ventricular systolic pressure, moderate or slight lowering of right ventricular systolic pressure, but little change from control values in either right or left initial tensions.

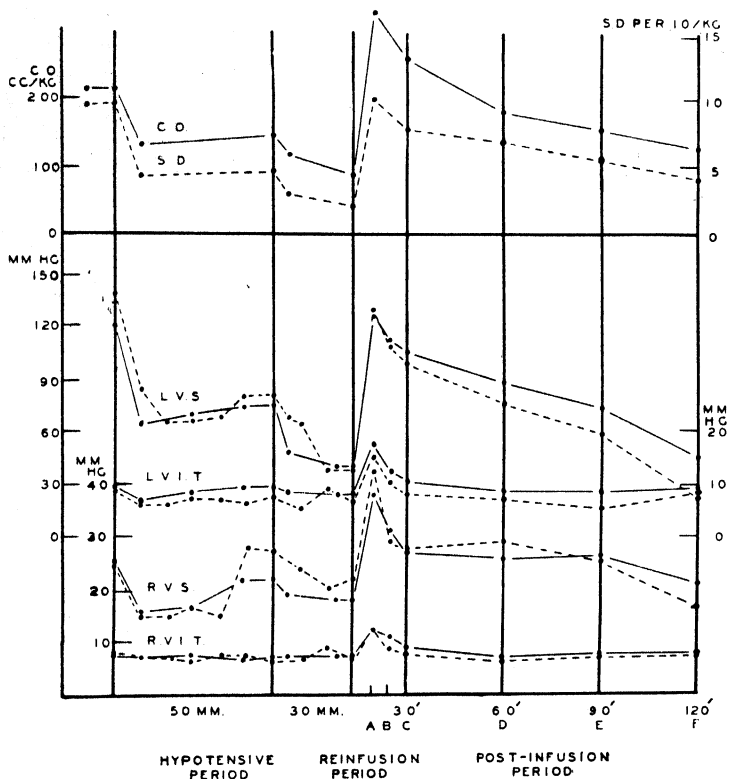


Fig. 4. Plots showing trend of changes in cardiac output (CO), systolic discharge (S.D.), initial tension in left ventricle (LVIT), and right ventricle (RVIT), systolic maximum in left ventricle (LVS), and right ventricle (RVS), during hemorrhagic hypotension, reinfusion, and subsequent circulatory failure. Discussion in text.

Reinfusion period. Reinfusion of all withdrawn blood results in a marked increase in all pressures (see also fig. 3). One interesting feature is the fact that initial tension in the left ventricle rises to a greater height than does initial tension in the right (17.4 mm. compared to 12.5 mm. Hg in figure 4).

Post-infusion circulatory failure. This period is characterized by a progressive fall in left ventricular systolic pressure, a fall and subsequent rise of left ventricular initial tension to slightly above the control level, maintenance of the right systolic pressure around normal and, finally, a rise slightly above control values

in right initial tension. However, this occurs only in the late stages when circulatory failure is well established. In experiment 86 the left ventricular initial tension did not rise above the control level in the late post-infusion period. Right systolic pressure declined only after circulatory failure had intervened. The latter finding was observed in all five experiments which progressed to the shock state.

DISCUSSION. In experiments on shock requiring prolonged periods of observation it has so far proved impossible to record changes in systolic discharge and minute output of the ventricles simultaneously with ventricular pressures. However, when similar procedures—such as our method for producing standard periods of hemorrhagic hypotension in a dog—are employed and they produce reasonably equivalent circulatory failure after reinfusion of withdrawn blood, comparison of results obtained in experiments performed in similar ways and in the same laboratory are perhaps permissible.

In this instance we have elected to compare changes in cardiac output per beat and per minute obtained in opened chest experiments by Wiggers and Werle (6) rather than results obtained on naturally breathing dogs by H. C. Wiggers (7). Average results of five such experiments are also plotted in figure 4.

Conclusive evidence that the right and/or left ventricle is depressed functionally would be furnished if it were found either during hemorrhagic hypotension or during post-infusion circulatory failure that *initial tension exceeded control values while the systolic ventricular pressure and systolic discharge are reduced*. Figure 4 shows that such relations exist only during the period *C-D*. Probable or suggestive evidence for myocardial depression would be furnished if, during periods of obvious reduction in systolic discharge, initial tensions remained at or returned to control levels. The data in figure 4 show that during the 50 and 30 mm. Hg periods of hypotension initial tensions are generally below control values. Exceptions sometimes occurred toward the end of the 50 and 30 mm. Hg periods respectively, i.e., initial tensions were sometimes restored to control values or slightly exceeded them. A review of cardiometer and pressure curves shows, however, that this generally takes place when the heart decelerates and the stroke volumes are mechanically increased. The greatest elevations of initial tension occur immediately after reinfusion of blood (*A*), and this elevation, though decreasing, may be maintained for 20 to 30 minutes (*B-C*). During the period *C-D*, cardiac output, systolic discharge, and the ventricular pressure maxima decrease rapidly despite the fact that initial tensions in the right and left ventricles are still above control values.

During the stage of progressive circulatory failure (*D-E*), arterial pressures decline, pulse pressure is reduced, systolic discharge of the ventricles is decreased below control values and the minute output declines markedly.

In the right ventricle initial pressure is a little less than in control curves, but the systolic pressure developed is not reduced as much as one would expect. Indeed, in one experiment charted as dotted lines the systolic pressure was greater

than normal. This suggests possible changes in resistance within the pulmonary circuit. It may be added parenthetically that no evidence of embolism or edema was found at autopsy in these experiments.

In the left ventricle, the initial tension was reduced only slightly at *D* and returned to control levels at *E* in average curves; occasionally it was elevated slightly. Taken in conjunction with the reduction in systolic pressure and the great decrease in systolic discharge this might be regarded as evidence that the ability of the left ventricle to respond to identical initial tension and diastolic stretch had been reduced. However, in the individual experiment 86, plotted, initial tension also decreased significantly at *D* and *E*.

In summary, the fact that initial tension in the right ventricle is less than control values during hemorrhagic hypotension and post-infusion shock would seem to eliminate right-sided failure as a factor in production of circulatory failure. There is a suggestion that the reaction of the left ventricle to equivalent initial tension is reduced during circulatory failure (*D-F*), but in view of the small differences in measurements one could wish that the evidence were more conclusive.

The foregoing interpretation of correlated data is of course predicated upon the assumption that, under conditions of our experiments, changes in diastolic size and fiber length are reflected in and correspond to changes in initial tension in the right and left ventricles. The question remains, however, whether initial tensions are reliable indices of diastolic stretch. It will be recalled that in their early publications Patterson, Piper and Starling (8) believed that no necessary relation existed. However, one of us (9) in numerous studies was unable to establish any dissociation. He was forced to conclude (1) that under a wide range of experimental conditions the diastolic size of the ventricles is fundamentally determined by the pressure created by inflowing blood, i.e., by the initial tension, and (2) that the conclusions of Patterson, Piper and Starling resulted because, at the time they worked, these investigators were not cognizant of the experimental possibility that the entire pressure curve may shift, and that conclusions cannot be drawn without registration of base lines and repeated static calibrations. However, conditions can occur in which such relations apparently do not hold, e.g., after premature systoles (10), following injection of epinephrine (11), during anoxia (12), during acute cardiac failure (13), and during pericardial effusion (14, 15). The possibility that the rate of ventricular relaxation after each stroke, or the degree of relaxation, i.e., cardiac tonus, may alter independently when reduced rather than increased venous return is occasioned, has not been studied thoroughly and must certainly be kept in mind.

The question must also be considered whether the reaction of the myocardium is as strictly related to initial tension and length as commonly assumed. Thus Warren et al. (16) working on human subjects presented evidence that cardiac output is not significantly affected by moderate hemorrhage although mean atrial pressure is markedly decreased. Such results on man must be accepted with caution for (1) since moderate hemorrhages reduce intrathoracic pressure, the effective venous pressures may not have changed in their studies, and (2) no

detail is given as to what points on varying venous pressure curves recorded from the right atrium were chosen for comparative measurements. The difficulties in such measurements have been discussed in several recent papers (6, 17). Nevertheless, the question propounded deserves consideration, for it is conceivable that changes in aortic pressure, in action of the ventricles, or in coronary vessels after bleeding may affect the ability of the heart to respond, even at the same initial tension. In other words, it seems to be increasingly more probable that changes in initial tension are an important but not the sole determinant of myocardial response.

SUMMARY

Right and left ventricular activities were studied by two methods in an effort to evaluate cardiodynamic factors involved in the progressive circulatory failure characteristic of hemorrhagic shock.

The first method consisted of recording right ventricular pressure curves in naturally breathing, anesthetized dogs optically by aid of a sound introduced into the right ventricle via a jugular vein. Left ventricular activity was inferred from aortic pressure curves. Successive right ventricular pressure curves recorded during hemorrhage and hypotension showed a more gradual rise, earlier summits and an abbreviation of the systolic ejection phase. Initial tension fell during hemorrhage, but increased during the latter part of the hypotensive period. Following reinfusion of all withdrawn blood the right ventricular pressure curves were restored to normal amplitude, slope and duration. During the period of progressive circulatory failure the transformation of the pressure curves resembled that seen during hemorrhage and hypotension. Shortening of systole occurred in the right ventricle as well as in the left ventricle as indicated by the aortic pressure curves both during hemorrhage and post-infusion failure. Such abridgement of systole was independent of heart cycle length and strongly suggests myocardial failure.

A more critical evaluation of initial tension changes was made by recording right and left ventricular pressure directly in open-chest dogs by means of cannulae passed through the myocardium into the ventricular chambers. This method avoided the effects of changing intrathoracic pressure. The results of these experiments confirm and extend the observations of the previous series. A small rise of right ventricular initial tension and a slightly greater rise of left ventricular initial tension in the late stages of shock does not appear to be significant when considered in conjunction with data on cardiac output and systolic discharge from comparable experiments. The question of whether initial tensions are reliable indices of diastolic stretch, and whether other determinants of myocardial response must be recognized, is discussed.

REFERENCES

- (1) WIGGERS, C. J. *This Journal* 144: 91, 1945.
- (2) WIGGERS, C. J. AND J. M. WEBER. *Proc. Soc. Exper. Biol. and Med.* 49: 604, 1942.
- (3) WIGGERS, C. J. *This Journal* 144: 91, 1945.
- (4) BROOKHART, J. M. AND T. E. BOYD. *Fed. Proc.* 5: 11, 1946.

- (5) WIGGERS, C. J. AND L. N. KATZ. This Journal **53**: 49, 1920. KATZ, L. N. AND H. FEIL. Arch. Int. Med. **32**: 673, 1923; **33**: 321, 1924.
- (6) WIGGERS, C. J. AND J. M. WERLE. This Journal **136**: 421, 1942.
- (7) WIGGERS, H. C. This Journal **140**: 519, 1944.
- (8) PATTERSON, S. W., H. PIPER AND E. H. STARLING. J. Physiol. **48**: 357, 1914.
- (9) WIGGERS, C. J. The pressure pulses in the cardiovascular system. Pp. 126, Longmans, Green and Co., London and New York, 1928.
- (10) WIGGERS, C. J. This Journal **73**: 346, 1925.
- (11) WIGGERS, C. J. AND B. STIMSON. J. Pharmacol. and Exper. Therap. **30**: 251, 1927.
- (12) STRUGHOLD, H. This Journal **94**: 641, 1930.
- (13) JOHNSON, V. AND L. N. KATZ. This Journal **118**: 26, 1937.
- (14) ADCOCK, J. D., R. H. LYONS AND J. B. BARNWELL. Am. Heart J. **19**: 283, 1940.
- (15) FINEBERG, M. H. Am. Heart J. **11**: 748, 1936.
- (16) WARREN, J. V., E. S. BRANNON, E. A. STEAD, JR. AND A. J. MERRILL. J. Clin. Investigation **24**: 337, 1945.
- (17) WIGGERS, C. J., D. F. OPDYKE AND J. R. JOHNSON. This Journal **146**: 192, 1946.

STUDIES ON THE BIOLOGICAL EFFECT OF HIGH FREQUENCY RADIO WAVES (RADAR)

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Soon after radar (radio detecting and ranging) equipment came into general use in World War II, rumors began to circulate that deleterious effects were being produced among personnel routinely exposed to its high-frequency, short wave length radiations. Although field investigations cast doubt that radar waves were a dangerous form of electro-magnetic radiation, rumors persisted that sterilization or alopecia might result from such exposure. Inasmuch as this portion of the electro-magnetic spectrum had not been explored biologically and because of the possibility that unshielded x-rays were emanating from the apparatus, the following experiments were undertaken by the Aero Medical Laboratory at the request of Hq AAF, Director of Communications.

MATERIALS AND METHODS. A radar sending unit, designated as Model no. SCR 517-A, was used as the source of the radiations. This unit has a peak output of 45 KW. The wave length of the rays produced is 10 cm. The paraboloid antenna was placed 2 feet away from a stand which was divided into compartments in which the experimental animals were placed. Some of the compartments were shielded with copper sheeting to exclude the radar radiations. A recording device, consisting of a neon bulb and photo-electric cell, was placed in front of the source of rays and was connected to an amplifier and electric pen recorder which gave a continuous record in order to show whether the apparatus was in operation at all times during each exposure period.

Thirteen male guinea pigs were exposed to the radiations for 3 hours daily for 51 to 53 days. Four of these animals were shielded at all times by copper sheeting. Five male guinea pigs served as controls, being kept in the same room but shielded and away from the source of the radar waves. The animals were weighed weekly; rectal temperatures were recorded for a one-week period before and after exposure to the radiations. At the conclusion of the experiments the males were mated to normal females. Following this the animals were sacrificed with ether. Blocks were taken from all organs; the tissues were fixed in Zenker formol, embedded in paraffin, and stained with hematoxylin and eosin. The following tissues were examined: heart, lung, liver, spleen, pancreas, adrenal, kidney, bladder, testis, epididymis, seminal vesicle, stomach, small intestine, neck organs (trachea, esophagus, thyroid, parathyroid), brain, eye, skin, muscle, and rib (costochondral junction and marrow).

EXPERIMENTAL RESULTS. *Appearance and growth.* The animals exposed to

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the radiations appeared normal in all respects. Growth was not impaired; the average total gain in weight for the exposed groups was 187.5 grams, while that of the controls was 174.8 grams.

Temperature. There was some temperature fluctuation among both the exposed animals and their controls; this was due to environmental changes. No difference could be detected in the former group as a result of the exposure.

Reproduction. The eight males which had been exposed to the radar waves daily for 51 to 53 days were each mated with a normal adult female. Of these eight females, seven gave birth to normal litters.

Histological changes. No abnormalities were found microscopically in the tissues which were examined. Particular attention was given to the testes, where spermatogenesis was found to be normal in each animal.

Shielded group. The growth, appearance, reproduction and tissues of the group shielded from the radar waves by copper sheeting were entirely normal. This was taken as evidence that x-ray radiations in any quantity were not being produced by the apparatus.

DISCUSSION. The effects of electro-magnetic radiations of certain wave lengths have been extensively studied. That tissue changes may be produced by waves of the ultraviolet and x-ray portions of the spectrum is well recognized. The experimental effects of visible and infra-red radiations have not been studied extensively; therefore, their physiological effects are less well understood. When one considers the portion of the spectrum beyond the infra-red, that of micro radio waves, no experimental data are available. To be sure, a large literature has accumulated on the biological effects of short-wave diathermy; in this procedure, however, the organism or its parts are placed directly in the electro-magnetic field, i.e., between the poles of the apparatus, so that the situation is very different.

There is little in the literature on the effects of high frequency radio waves on the organism. Apparently because of a somewhat similar morale problem, Daily of the Naval Research Laboratory (1) studied a group of personnel exposed to radar radiation for periods up to nine years. No effects were noted on blood hemoglobin or white cell and differential counts. Reproductive function was apparently not altered, nor was there any evidence that exposure led to alopecia. There was a certain number of instances of frontal headache and intra-ocular pain, occurring after several hours of exposure; these symptoms disappeared shortly after exposure was terminated. Other personnel described flushing of the face and a feeling of warmth in the hands when these portions of the body were placed directly in the field.

Another study has been reported by Lidman and Cohn (2). These investigators examined a group of Air Force personnel exposed to the emanations of radar for prolonged periods. No evidence of stimulation or depression of erythropoiesis or leukopoiesis could be demonstrated.

SUMMARY

Guinea pigs were exposed to ultra short wave radiations (radar) for three hours daily for eight weeks. No effects were noted on the appearance, growth, body

temperature or reproduction. Histological examination of the tissues failed to reveal any changes.

I wish to express my appreciation to Colonel Otis O. Benson, M.C., under whose supervision this work was initiated.

REFERENCES

- (1) DAILY, L. E. U. S. Nav. Med. Bull. **41**: 1052, 1943.
- (2) LIDMAN, B. I. AND C. COHN. Air Surgeon Bull. **2**: 448, 1945.

THE EFFECT OF TIME OF DAY ON THE METABOLIC RATE OF ALBINO MICE; A MANOMETRIC METHOD¹

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Measurements of metabolic rates of small animals have usually been made by placing several animals in one chamber and determining average oxygen consumption or carbon dioxide production (cf. Rubner, 1902; Benedict and Fox, 1933; Herrington, 1940). Implicit in this method is error in the calculated metabolic rate of each animal due to individual differences such as body size and the degree of activity during the determination. It is evident that individual determinations give more useful results than average figures obtained from group observations (cf. Scheff and Rabati, 1938).

The apparatus described below is so constructed that each animal occupies a separate chamber. Readings are taken by means of individual manometers and observations on weight, activity and body temperature are made on each animal individually. An apparatus similar to ours has been described by Issekutz and Novak (1936).

The data reported here give normal metabolic rates for mature albino mice which were neither fasted nor anesthetized. Since no attempt was made to determine a *basal* rate, values shown here are somewhat higher than the few comparable ones in the literature. Experimental conditions have been similar to ours in only a few studies (cf. Benedict and Fox, 1933). Aszódi (1921) recorded a value for fasted mice of 2.13 ml. oxygen per gram per hour at 29–30°C. Davis and Van Dyke (1933) reported that at 28°C. mice used 1.60 ml. oxygen per gram per hour. Boettiger (1941) found that young fasted mice used 2.34 ml. oxygen per gram per hour at 33°C. Scheff and Rabati (1938), using a manometric apparatus, reported a value of 2.16 ml. oxygen per gram per hour at 28°C. Since the purpose of the experiments herein reported was to determine the cause of the apparent variation in metabolism of the mice during the day, it was essential that the activity and feeding of the animals be normal. The “morning” and “afternoon” values thus obtained have been treated statistically to determine the factors which influence this variation and to estimate their relative importance.

METHODS. The apparatus used in these experiments² is a modification of the Warburg constant volume type manometric apparatus and permits the measurement of rates as well as total amounts of oxygen consumed. The animal chamber consists of a glass vessel of about 500 ml. volume connected to a mercury-filled manometer tube. A similar empty vessel serves as a thermobarometer. The mercury in the tube is adjusted to keep the gas at constant volume and the change

¹This work was aided by a grant from the Research Fund of the Stanford University School of Medicine.

²Constructed by the E. Machlett Co., New York.

in pressure is read on the open arm of the U-tube, which is graduated in millimeters. The vessel is connected to the manometer tube by a ground-glass joint sealed with "Lubriscal" and held firmly in place by rubber bands. Each vessel is equipped with glass supports 4 cm. high, on which is placed a round copper screen retained by a piece of rubber-covered flexible wire. The animal sits on this platform out of contact with the sodium hydroxide solution. During the experimental period the vessel is completely immersed in a water thermostat which is regulated at $T^\circ \pm 0.1^\circ\text{C}$. The tank is constructed with one side of glass so that the animals may be observed continually and their activity recorded. The vessels were calibrated (using water) and the constants calculated by the method described by Dixon (1943).

Ten milliliters 25 per cent NaOH (pipetted into the bottom of the vessel) was found to be adequate for CO_2 absorption. Each mouse was weighed to 0.1 gram before being placed in the chamber; care was taken so that the animal was not

TABLE 1

-1 animal sleeping
0 animal wake, but not moving
1 animal moving head and neck; otherwise quiet
2 animal moving about in vessel; exploratory movements; or standing on hind legs
3 animal very active; the maximum activity possible in the vessel

excited unnecessarily. The vessels (six) were flushed with oxygen before the ten-minute equilibration period in the thermostat. Readings were taken every five minutes for at least one hour, and at each reading the degree of activity was observed and recorded on an arbitrary scale, shown in table 1, with 0 corresponding approximately to the conventional repose of basal metabolism. Uniformity of grading activity was favored by having all records made by one person.

The total cumulative oxygen consumed was plotted against time: the points fell on a straight line in every case except when the activity of the mouse changed noticeably during a part of the period measured. The oxygen consumption has been expressed as a weight-specific rate, here denoted by the symbol Q_{O_2} , which is defined as the milliliters of oxygen consumed (at STP), per hour per gram of animal weight, following the terminology used by Warburg for isolated tissues (Warburg, 1930). The metabolic rate is obtained by substitution in the following formula:

$$Q_{O_2} = \frac{(V_G - W) K R}{W}$$

in which

V_G = gas volume of vessel (ml.) containing CO_2 absorbent and platform,

W = weight of animal in grams. (To correct for volume occupied by mouse, the specific gravity of the animal assumed to be 1.)

$K = \frac{273}{t \bar{P}_0}$ where P is normal pressure of manometric fluid in cm. Hg. Amount of dissolved O_2 negligible, and not included in calculation of K .

R = change in manometric reading in cm. during 1 hour.

Fifteen pure-strain albino mice were used. Fifty-nine determinations of metabolic rate were made with all experimental conditions constant except the time of day at which the measurement was made. All experiments were done with the thermostat at 30°C. (thermal neutrality for the mouse; Chevillard, 1935). Experiments designated "a.m." were performed from 10 to 11 o'clock in the morning, and those designated "p.m." were performed from two to three o'clock in the afternoon, Pacific War Time.

RESULTS AND DISCUSSION. In table 2 the mean Q_{O_2} and activity are given for the groups indicated. The correlation between Q_{O_2} and activity for the 59 individual determinations is 0.814, with a standard error of estimate of the regression of Q_{O_2} on activity of 0.626. The slope of the line (calculated by the method of least squares) obtained is 0.715 and the intercept 2.551.

TABLE 2
*Mean oxygen consumption rate (Q_{O_2}) and mean activity at 30°C.
morning and afternoon values*

GROUP	NO. MICE	EXP. NO.	TIME OF DAY	MEAN Q_{O_2}	MEAN ACTIVITY
I (Males)	5	1	p.m.	2.45	0.12
	5	2	a.m.	3.94	2.20
	5	3	p.m.	1.93	-0.30*
	4	4	p.m.	3.21	1.40
II (Females)	5	1	p.m.	3.78	0.90
	5	2	p.m.	3.79	0.70
	5	3	a.m.	4.12	1.20
	5	4	a.m.	4.03	2.00
III (Males)	5	1	a.m.	4.01	1.90
	5	2	a.m.	3.99	1.80
	5	3	p.m.	2.11	-0.50
	5	4	p.m.	2.19	0.50

* Activity which is (-) denotes sleeping condition for part of experimental period (cf. table 1).

When the morning and afternoon Q_{O_2} values are averaged separately there is a statistically significant difference between them. The average morning Q_{O_2} and activity are 4.02 and 1.80 respectively. The afternoon averages are 2.78 and 0.40 for Q_{O_2} and activity. The differences in Q_{O_2} were shown to be significant by Fisher's method of analysis of variance (Fisher, 1936). The value of P_t was less than 0.001. Differences caused by individual variation (e.g., sex and body size) were not significant.

Terroine and Troutmann (1927) have shown a diurnal variation in the body temperature of mice. However, rectal temperatures of the mice in our study (determined by means of a thermocouple) showed little difference between average morning body temperature (37°C.) and average afternoon body temperature (36.7°C.). Since this difference is not significant, chance variation in body temperature is apparently not the cause of the morning-afternoon variance in Q_{O_2} .

Horst, Mendel and Benedict (1934a) found the oxygen uptake of rats higher in the morning and after 4 p.m. than during the middle of the day. These authors (1934b) also reported that at 30°C. rats showed an increased rate of oxygen consumption after exercise. A direct relationship between activity and metabolic rate has been reported by others (Benedict, 1936; Brody and Cunningham, 1936; Davis and Van Dyke, 1932). The correlation obtained above (0.814) between activity and Q_{O_2} would indicate that some 66 per cent of the variance in Q_{O_2} may be explained by variance in activity. Since there is evidence (Wolf, 1930) that mice are more active in the morning than during the afternoon, the morning-afternoon difference in Q_{O_2} could be explained on the basis of the difference in activity.

TABLE 3

Significance of differences between a.m. and p.m. values for oxygen consumption rates

GROUP NO.	TIME OF DAY	NUMBER OF DETERMIN.	E	P	C	P
II	p.m.	10	3.78	0.0001	3.25	0.47
II	a.m.	10	4.08		2.91	
III	p.m.	10	2.15	0.0001	2.17	0.54
III	a.m.	10	4.00		2.69	

E is experimentally determined Q_{O_2} .

C is calculated Q_{O_2} at "zero" activity.

P is the probability that differences as great or greater than those observed between morning and afternoon values would occur through random sampling.

To exhibit the effect of such activity, a correction factor was applied to the observed values of metabolic rate in groups II and III. The results of these calculations are shown in table 3. Each Q_{O_2} was reduced to "zero" activity, the corrected value of Q_{O_2} (*C*) resulting from substitution in the following formula:

$$C = E - (b A)$$

in which

C is the calculated Q_{O_2} at "zero" activity

E is the experimentally determined Q_{O_2}

b is the regression of Q_{O_2} on activity

A is the activity observed.

Since only observable activity is recorded, the "corrected" values do not include correction for changes in skeletal muscle tonus.

The differences between the uncorrected morning and afternoon Q_{O_2} values are statistically highly significant (*P* is less than 0.001). But when these values are compared after the oxygen cost of the observed activity has been subtracted, the differences are not significant; the mean Q_{O_2} corrected for activity is 2.80 for a.m. and 2.71 for p.m. (*P* is 0.4 to 0.5). This type of activity record serves then as a reliable estimation of activity levels and may be used in metabolism studies where variability in activity is encountered.

SUMMARY

1. A manometric apparatus for the measurement of individual metabolic rates of small animals is described.

2. Rates of oxygen consumption (Q_{O_2}) of albino mice were determined during the morning and afternoon and the activity of the animals recorded. A correlation of 0.8 was found between activity and Q_{O_2} . Statistical analysis showed the difference between morning and afternoon oxygen consumption rates to be highly significant, but when these rates were corrected for the oxygen cost of observable activity, the difference was *not* significant.

3. Although many factors affect the level of metabolism in mice, the present observations indicate that, at thermal neutrality, the variation in observable activity is the dominant element.

The authors wish to acknowledge the contributions to this work of Dr. V. E. Hall in developing the apparatus, of Dr. F. W. Weymouth for aid in statistical analyses and of Drs. J. M. Crismon and John Field for helpful suggestions.

REFERENCES

- AZÓDI, Z. Biochem. Ztschr. **113**: 70, 1921.
 BENEDICT, F. G. Carnegie Inst. of Wash., Publ. no. 503, 1936.
 BENEDICT, F. G. AND L. FOX. Pflüger's Arch. **231**: 455, 1933.
 BOETTIGER, E. G. Endocrinology **28**: 785, 1941.
 BRODY, S. AND R. CUNNINGHAM. Res. Bull. Univ. Missouri Agric. Expt. Station no. 244, 1936.
 CHEVILLARD, L. Ann. Physiol. Physicochim. biol. **11**: 461, 1935.
 DAVIS, J. E. AND H. B. VAN DYKE. J. Biol. Chem. **95**: 73, 1932.
 J. Biol. Chem. **100**: 455, 1933.
 DIXON, M. Manometric methods. The University Press, Cambridge, 1943.
 FISHER, R. A. Statistical methods for research workers. Oliver and Boyd, London, 1936, p. 214.
 HERRINGTON, L. P. This Journal **129**: 123, 1940.
 HORST, K., L. B. MENDEL AND F. G. BENEDICT. J. Nutrition **7**: 277, 1934a; J. Nutrition **7**: 251, 1934b.
 ISSEKUTZ, B. V. AND E. NOVÁK. Tagung der Ungarischen Physiologischen Gesellschaft, Tihany. 1936. Ber. ges. Physiol. **94**: 672, 1936.
 RUBNER, M. Die Gesetze des Energieverbrauchs die der Ernährung. Leipzig, 1902.
 SCHEFF, G. AND F. RABATI. Biochem. Ztschr. **298**: 101, 1938.
 TERROINE, E. F. AND S. TRAUTMANN. Ann. Physiol. Physicochim. biol. **3**: 422, 1927.
 WARBURG, O. Metabolism of tumours. Transl. by F. Dickens. Smith, New York, 1930.
 WOLF, E. Ztschr. vergl. Physiol. **11**: 321, 1930.

THE GENERAL TOLERANCE AND CARDIOVASCULAR RESPONSES OF ANIMALS TO EXPLOSIVE DECOMPRESSION¹

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It is generally agreed that the most satisfactory solution to many of the physiological problems of high altitude flight is the pressurized cabin. However, as is so often the case, a solution to one problem introduces others. Thus, when the pressurized cabin comes into general use, anoxia and decompression sickness will cease to be problems, but the physiological effects of rapid decompression such as would occur in case of mechanical failure, the blowing out of a port, or the piercing of the pressurized cabin with a large caliber projectile will become of paramount importance.

Prior to 1942, the available literature on the subject of explosive decompression was extremely scanty. Armstrong (1939) reported a few experiments, and Hoff and Fulton (1942) list some half dozen pertinent references.

In the summer of 1942, we began in this laboratory an extensive study of the physiological and pathological effects of explosive decompression. These studies have continued to the present time.

In this paper we are reporting the general responses and tolerance of various species of laboratory animals to explosive decompression, including cardiovascular responses of anesthetized dogs.

METHODS. Explosive decompressions were effected in the following manner. A large decompression chamber with a volume of approximately 12.74 cubic meters (450 cu. ft.) was evacuated to any desired barometric pressure. Explosive decompression of a smaller animal chamber could then be obtained by suddenly completing a connection between this chamber and the larger one. The range of decompression was determined by the pressure differential existing between the two chambers, prior to the establishment of the connection, and the rate of the decompressions varied with the volume of the small chamber and the size of the connecting opening. Three decompression systems based on these principles have been used.

In the first of these, the animal chamber has a volume of 1.19 cubic meters (42 cu. ft.). It is connected to the large decompression chamber by 13 feet of 1.5 inch pipe and 27 feet of 2 inch pipe. The connection is completed by means of a knife valve near the small chamber. We shall call this System A.

In the second system, the animal chamber consists of a glass bell jar with a volume of about 22.7 liters (0.8 cu. ft.). Connection with the large tank is

¹ The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Ohio State University Research Foundation.

made by means of 13 inches of 1 inch pipe and a knife valve. We shall refer to this as System B. The size of the bell jar prevents the use of large animals with this system.

In the third system used, the animal chamber is a steel tank having a volume of 0.212 cubic meter (7.5 cu. ft.). It is connected to the large chamber by means of an opening 15 inches in diameter, which is provided with a machined flange.

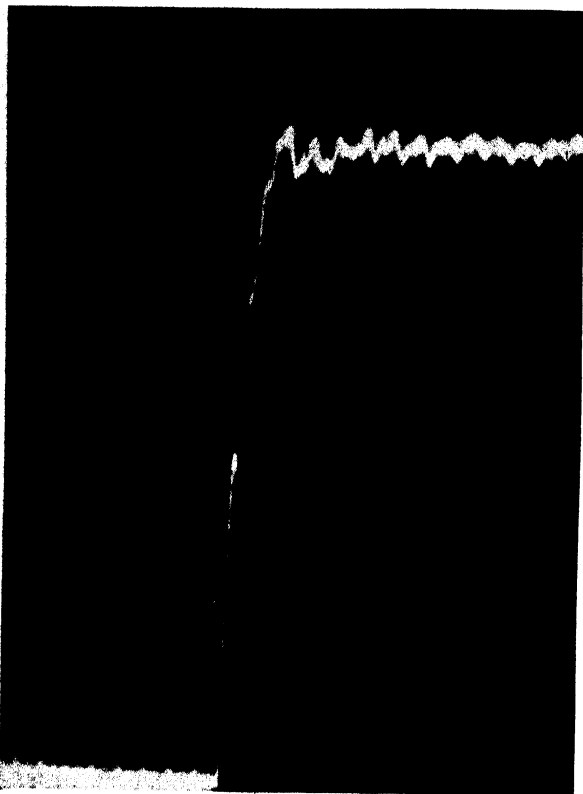


Fig. 1. Time-pressure curve for explosive decompression with System C and the 15 inch aperture.

Time intervals $1/120$ sec. The beam rises with decreasing pressure.

Total time from 750 mm. Hg (ground level) to 87 mm. Hg (50,000 ft.) is 0.02 second.

This flange is bolted to a similar flange which has been welded to a 15 inch opening in the large decompression chamber. A sheet of cellulose acetate 0.02 inch in thickness is placed between these two flanges and the bolts tightened up so as to make an air-tight connection. The desired pressure gradient is then established between the two chambers. Shearing out of the membrane, with the resulting rapid equalization of pressure, is obtained by burning a slit in the membrane with an electrically heated loop of nichrome wire. We shall call this System C. The rate of decompression with this system can be varied by the

use of a series of four metal plates designed to fit the opening between the chambers and provided with apertures of 3, 6, 8 and 10 inch diameters respectively. Five rates of decompression are thus obtainable with this system.

The decompression rates obtained with these systems have been measured by means of calibrated, optically recording rubber membrane or glass spoon manometers. A typical record for System C with the 15 inch aperture is shown in figure 1. As is to be expected, the curve shows that the decompression takes place at a constantly changing rate. It should be kept in mind, therefore, that

TABLE 1

Average rates of pressure changes with the described decompression systems

SYSTEM	A	B	C				
			Diameter of aperture in inches				
			15	10	8	6	3
Rate mm. Hg/sec.....	26.9	1124	33,650	13,460	8520	4800	1200
Rate lbs. per sq. inch/sec.....	0.52	21.7	650	260	165	93	23

TABLE 2

Summary of experiments

	SYSTEM A	SYSTEM B	SYSTEM C				
Decompression rate, mm. Hg/sec..	26.9	1124	1200	4800	8520	13,460	33,650
No. of animals.....	18	82	20	61	11	18	100
No. of experiments.....	40	235	44	146	21	29	194
No. of experiments with anesthesia.....	28	92	44	141	21	26	133
No. of experiments without anesthesia.....	12	143	0	5	0	3	61
Rats.....	0	16	0	0	0	0	2
Guinea pigs.....	3	35	0	0	0	0	5
Rabbits.....	7	17	1	2	0	2	5
Cats.....	7	13	0	0	0	2	2
Dogs.....	1	1	19	59	11	14	86

while we have established rates of decompression in terms of an average based on the time required for a pressure change of a given magnitude to take place, animals undergoing such decompression are subjected to a constantly varying rate of pressure change. The average rates of pressure change with the systems used are given in table 1. Rates of decompression are expressed in terms of millimeters mercury per second. Equivalent values in terms of pounds per square inch are also included.

Since these studies were primarily concerned with the physiological effects of explosive decompression itself, an attempt was made to minimize the development of coincident anoxia. The experimental animals were therefore rapidly recompressed to normal barometric pressures within one to two minutes after

the explosion, and the animal chambers were flooded with oxygen at the moment of decompression.

Nembutal, in doses of 30 mgm. per kilogram of body weight, intraperitoneally, was used where anesthesia was required.

In experiments on cardiovascular responses, blood pressures were recorded directly from a carotid artery either with the usual mercury manometer, or with an optically recording membrane manometer.

The data on general responses and tolerance are based on a total of 709 explosive decompressions. The distribution of these experiments with regard to rate of decompression, number and species of animal used and presence or absence of anesthesia, is presented in table 2. The bulk of these decompressions were performed from initial pressures of about 750 mm. Hg (ground level) or 522 mm. Hg (10,000 ft.) to final pressures of 141 mm. Hg (40,000 ft.) or 87 mm. Hg (50,000 ft.). In 44 experiments the final pressure was 54 mm. Hg or less (altitude of 60,000 ft. or above).

Observations on cardiovascular responses were made in 93 experiments on 70 anesthetized dogs.

RESULTS. A. General Response and Tolerance. The laboratory animals used showed excellent tolerance to the rates and ranges of decompression investigated. In no case did an animal die as the result of a single decompression, or as the result of multiple decompressions performed in the course of a single experiment, providing that recompression was rapidly carried out. This finding applied even to the extremely drastic experiments in which the final pressure was 54 mm. Hg or less.

The outstanding and most consistent response observed in these animals was abdominal distention. While no quantitative measurements were made, the extent of this distention appeared to vary with the species of animal used, while within a single species there seemed to be an inverse relation to the final pressure attained. Abdominal distention was greatest, as might be expected, in the rabbit and guinea pig, due to the relatively large amounts of gas normally present in the gut of these animals. It persisted until recompression of the animal. The degree of abdominal distention seemed to be independent of the rate of decompression. This distention probably has an important effect on the mechanics of respiration. As the distention increases, the diaphragm is of necessity forced up into the expiratory position while the thoracic cage is lifted into the inspiratory position. In the rabbit and guinea pig these effects may be so pronounced as seriously to interfere with, or actually prevent, respiratory movements, and in all animals there is usually some interference with respiration.

Transient expansion of the thorax occurred in all animals explosively decompressed, but was largely masked by the abdominal distention.

Defecation and urination frequently occurred. Increased intestinal pressures and pressure on the bladder are undoubtedly important causal factors in the production of these reactions although fright, resulting in part from the noise associated with the decompression, may have played a contributing rôle.

Since experimental animals showed excellent tolerance to a single rapid de-

compression, or to repeated exposures in a single experiment, the effects of repeated exposures over a period of days were studied. Animals were decompressed once daily for from 5 to 11 days and then observed for at least two weeks following the final decompression. The experiments are outlined in table 3. There were 3 fatalities among the 25 animals observed. One of these, the guinea pig, was due to a rupture of the stomach, with generalized peritonitis, and was undoubtedly the result of explosive decompression. The cause of death in the case of the two cats was not clear. One animal, in apparently good health, was found dead on the twelfth day following the last decompression. The other seemed chronically ill for some days prior to death but had shown no definite localizing signs. Both deaths occurred in the summer months

TABLE 3

Tolerance to daily exposures to explosive decompression

A. Decompression rate 1124 mm. Hg/sec.

Decompression range 750 or 523 mm. Hg to 87 mm. Hg
(0 or 10,000 to 50,000 ft.)

SPECIES	NO. OF ANIMALS	AVERAGE NO. OF DECOMPRESSIONS	FATALITIES	CAUSE OF DEATH
Cat.....	5	11	2	Unknown Ruptured stomach
Guinea pig.....	2	6	1	
Rabbit.....	1	7	0	
Rat.....	6	6	0	

B. Decompression rate 33,650 mm. Hg/sec.

Decompression range 523 mm. to 87 mm. Hg.
(10,000 to 50,000 ft.)

SPECIES	NO. OF ANIMALS	AVERAGE NO. OF DECOMPRESSIONS	FATALITIES	CAUSE OF DEATH
Rat.....	7	7	0	
Dog.....	4	5	0	

and post mortem changes made the autopsies unsatisfactory. These fatalities are the only ones we have seen in the course of the 709 experiments reported.

Post mortem examinations of some 40 animals, sacrificed following decompressions with System B, were performed. The outstanding gross findings were small hemorrhagic lesions in the lungs, and in the walls of the gastrointestinal tract. The presence of these lesions, however, was not incompatible with the life of the animal, nor with apparently normal activity. In no case have we seen evidence of gas bubble formation in the blood stream. Further observations on the pathology of explosive decompression will be reported elsewhere.

B. *Cardiovascular Responses.* Dogs exposed to explosive decompressions of sufficient magnitude consistently showed significant drops in systemic blood pressure, sometimes accompanied by cardiac slowing.

1. *The nature of the blood pressure drop.* Figure 2 shows a typical blood pres-

sure curve, taken with the ordinary mercury manometer, during and following decompression from a pressure of 522 mm. Hg to 87 mm. Hg (10,000 ft. to 50,000 ft.) in 0.03 second. The rate of pressure change equaled 13,460 mm. Hg (260 psi) per second. Figure 2b shows the initial portion of a curve obtained with a

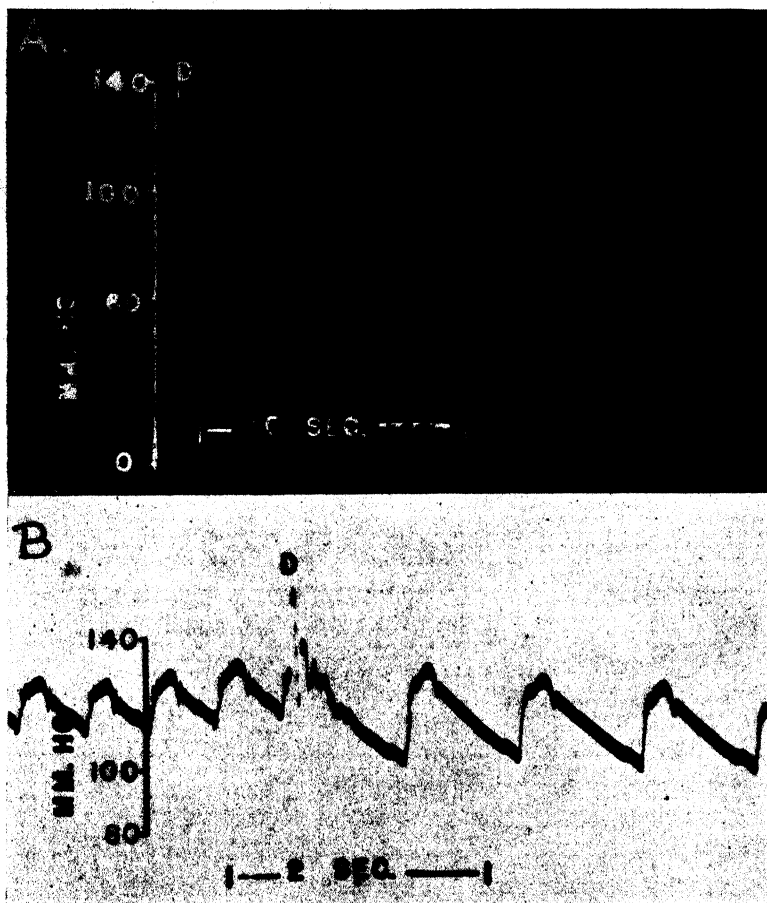


Fig. 2. A. Mercury manometer record of carotid blood pressure of a dog decompressed from 522 mm. Hg (10,000 ft.) to 87 mm. Hg (50,000 ft.) in 0.03 second. Decompression at D.

B. Electrical capacitance manometer record of carotid blood pressure. Range of decompression as in A, but time of decompression 0.01 second.

metal membrane manometer of the electrical capacitance type during decompression over the same range, but at a rate of 33,650 mm. Hg (650 psi) per second. Several features deserve mention.

First, the blood pressure does not begin to fall significantly for one or two heart beats following the instant of decompression.

Second, there is marked cardiac slowing, beginning at the instant of decompression.

Third, the blood pressure quickly returns to normal levels, the duration of the drop being less than a minute.

2. *The extent of the blood pressure drop.* The extent of the blood pressure drop varies with the rate and range of the decompression. Table 4 summarizes the responses of 45 animals to decompression over a constant range of 435 mm. Hg (10,000 to 50,000 ft.) at varying rates. It can be seen that there is no significant difference in the average drop at rates varying from 1,200 mm. Hg (23 psi) per second to 8,520 mm. Hg (165 psi) per second although the maximum drop tends to increase with increase in rate. With rates greater than these, however, the average drop is significantly increased. In any one animal subjected to decompressions at varying rates over a constant range, the extent of the blood pressure drop varies directly with the speed of the decompression. Rates of 1,200 mm. Hg (23 psi) per second and 4,800 mm. Hg (93 psi) per second sometimes do not induce any fall in blood pressure, and rates less than those listed are without effect in our experience.

TABLE 4

The extent of the blood pressure drop in animals decompressed from 522 mm. Hg (10,000 ft.) to 87 mm. Hg (50,000 ft.) at varying rates

RATE OF DECOMPRESSION	NO. OF ANIMALS	NO. OF OBSERVATIONS	MAXIMUM DROP	MINIMUM DROP	AVERAGE DROP	CARDIAC SLOWING
<i>mm. Hg/sec.</i>			<i>mm. Hg.</i>			
1,200	7	8	40	0	24	None
4,800	13	19	50	0	28	None
8,520	10	10	65	5	28	Occasional
13,460	10	10	80	32	50	Present
33,650	5	5	100	75	81	Present

If the rate of decompression is kept constant, the extent of the blood pressure drop is dependent on the range of decompression. The results of experiments on 29 animals are presented in table 5. The extent of the blood pressure drop varies directly with the range of the decompression. Ranges of less than 300 mm. Hg do not induce significant changes with this rate of decompression (4,800 mm. Hg per second).

Experiments of a similar nature, but using other rates of decompression, yield results indicating a reciprocal relationship between the rate and range necessary to produce a drop in blood pressure. The greater the rate of decompression, the smaller the range necessary to induce a blood pressure response, and vice versa. It is possible, therefore, even with the most rapid rates of decompression to find a range so small that no blood pressure drop occurs. Experiments indicate that with our fastest rate of decompression, 33,650 mm. Hg (650 psi) per second, a range of decompression of at least 200 mm. Hg is necessary for the production of a significant blood pressure drop.

3. *Cardiac slowing.* The occurrence of cardiac slowing is indicated in table 4. With rates of 13,460 mm. Hg (260 psi) per second or greater, significant brady-

cardia is consistently found. Bilateral vagotomy prevented the appearance of the bradycardia, but did not prevent the accompanying blood pressure drop, although it tended to reduce its severity.

4. *The effect of increased expiratory resistance.* In six experiments the animals breathed through tracheal cannulae provided with inspiratory and expiratory valves and with a device for varying the diameter of the expiratory opening. In all cases the blood pressure drop following explosive decompression was markedly greater when expiratory resistance was increased. Blood

TABLE 5

The effect of the range of decompression on blood pressure responses of animals decompressed at a rate of pressure change of 4,800 mm. Hg/sec.

RANGE	NO. OF ANIMALS	NO. OF OBSERVATIONS	MAXIMUM DROP	MINIMUM DROP	AVERAGE DROP
mm. Hg			mm. Hg		
275	5	5	12	0	3
300	5	5	12	0	9
375	5	5	25	0	14
435	13	19	50	0	28
673	1	1	70	70	70

TABLE 6

The effect of increased expiratory resistance on the blood pressure response to explosive decompression

Dog 1-12-43

RATE	RANGE	DIAMETER OF EXPIRATORY OPENING	FALL IN B.P.	CARDIAC SLOWING
mm. Hg/sec.	mm. Hg		mm. Hg	
4,800	522-87	Normal (15 mm.)	18	None
4,800	522-87	2.5 mm.	55	Present
4,800	522-87	Normal	10	None
4,800	522-87	Normal	0	None
4,800	522-87	1.0 mm.	60	Marked
4,800	522-87	4.5 mm.	45	Present

pressure drops of more than twice the normal value for a given rate and range, and marked cardiac slowing, could be produced by increasing the expiratory resistance. Results of a typical experiment are given in table 6.

DISCUSSION. The pressure of the atmosphere is exerted continuously and equally on all parts of the body. Since the body tissues are essentially fluid in character, any change in this pressure is transmitted equally and instantly throughout the body. Explosive decompression is thus without effect on the solid or fluid filled organs of the body, providing that the entire body is exposed to the pressure change. Gases contained in the hollow organs, however, will respond to changes in barometric pressure in accordance with the physical laws governing gas volumes. Explosive decompression, therefore, will result in a

tendency toward increased gas volumes in the two main gas-containing systems of the body, the respiratory and gastrointestinal tracts. If the contained gases are not free to expand, or if they cannot escape from the body, then varying degrees of increased pressure will occur in the organs involved.

The primary physiological changes occurring at the time of explosive decompression, therefore, are increased intra-abdominal and intrathoracic pressures. The extent of the increased intra-abdominal pressure will depend on the amount of gas in the gut and the final ambient pressure reached. It will persist until return to normal barometric pressure unless it is relieved by the escape of gas from the gastrointestinal tract.

The increase in intrathoracic pressure occurs when the ambient pressure falls at a rate greater than that at which air can escape from the lungs. Under such conditions the lungs dilate, the thorax is forcibly expanded, and the intrapulmonary pressure rises. This pressure, unlike the intra-abdominal pressure, depends on both the rate and range of the decompression, and persists for only a short time since equilibrium with the ambient atmosphere is soon established by the escape of air through the trachea. This intrathoracic pressure should be greater with greater rates of decompression at a given range, and greater with greater ranges of decompression at a given rate.

The data reported here indicate that experimental animals are highly tolerant to the development of these pressures. The one fatality undoubtedly related to explosive decompression, however, was the result of expansion of gas in the stomach and it is significant that such pathologies as have been found are localized in the pulmonary and gastrointestinal organs.

The effects on blood pressure and heart rate are, we believe, primarily the result of the rise in intrathoracic pressure. It has long been known that such a rise may produce a drop in systemic blood pressure. Interference with cardiac filling and output due to collapse of the great veins, "tamponade" effect of the distended lungs on the heart, changes in the pulmonary circulation and vagal cardiac and vasomotor reflexes from the lungs have been suggested by various authors as causal mechanisms (Humphreys, Moore and Barkley, 1939), (Johnson and Luckhardt, 1928), (Luckhardt and Johnson, 1928), (Visscher, Rupp and Scott, 1924). The operation of these mechanisms would satisfactorily explain the delay in the onset of the blood pressure drop since an effect on cardiac filling would not be manifested immediately. The vagal bradycardia observed with the faster rates is similar to that described by Luckhardt and Johnson (1928). The relationship of the drop to the rate and range of decompression parallels the expected variations in intrathoracic pressure. The results of the experiments with increased expiratory resistances, and, therefore, greater and longer lasting increases in intrathoracic pressures, constitute additional evidence in support of this opinion. Blood pressure drops of more than twice the normal values for a given rate and range, and cardiac slowing not normally seen, could be induced by increasing the expiratory resistance. The relationship between increased intrathoracic pressure, pulmonary distention and the effects described seems clearly established.

Since it has been shown (Pearcy and Van Liere, 1928) that distention of the gut, while not always a consistent stimulus, may induce reflex drops in blood pressure, this factor may play a contributory rôle.

While it might be expected that explosive decompression would offer a most favorable condition for supersaturation of blood and tissues with their dissolved gases, and consequently for resulting bubble formation, it can only be said that under the circumstance of these experiments such bubble formation was not observed.

SUMMARY

Methods for the production and measurement of explosive decompressions at a variety of rates and ranges are described.

Laboratory animals show excellent tolerance to the rates and ranges of decompression studied. However, certain pathologies, notably hemorrhages in the pulmonary and gastrointestinal systems, may occur.

Thoracic and abdominal distention are the outstanding responses to explosive decompression noted in all animals.

One fatality in 709 decompressions can definitely be attributed to explosive decompression.

Anesthetized dogs subjected to explosive decompressions of sufficient magnitude show a drop in systemic blood pressure.

The extent of the blood pressure drop varies directly with the rate and range of the decompression. Bradycardia of vagal origin accompanies the severe drops which follow decompressions at rapid rates over large ranges.

Increasing the resistance to expiration markedly increases the severity of blood pressure drops at a given rate and range of decompression.

Distention of the lungs and increased intrathoracic pressure, occurring when the rate of decompression of the chamber exceeds the rate at which the lungs can decompress, is considered to be the primary cause of the blood pressure drop.

REFERENCES

- ARMSTRONG, H. G. Principles and practice of aviation medicine. Williams & Wilkins, Baltimore, 1939.
- HOFF, E. C. AND J. F. FULTON. A bibliography of aviation medicine. Charles C. Thomas, Springfield, Baltimore, 1942.
- HUMPHREYS, G. H., R. L. MOORE AND H. BARKLEY. J. Thoracic Surg. 8: 553, 1939.
- JOHNSON, C. A., AND A. B. LUCKHARDT. This Journal 83: 642, 1928.
- LUCKHARDT, A. B. AND C. A. JOHNSON. This Journal 84: 453, 1928.
- PEARCY, J. F. AND E. J. VAN LIERE. This Journal 83: 445, 1928.
- VISSCHER, M. B., A. RUFF AND F. H. SCOTT. This Journal 70: 586, 1924.

THE ASSAY OF NEPHROSCLEROSIS PRODUCING ANTERIOR PITUITARY PREPARATIONS

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The production of nephrosclerotic lesions with desoxycorticosterone acetate (D.C.A.) in chicks, rats, dogs and monkeys has been clearly demonstrated in the experiments of Selye and co-workers (1-4). This effect of D.C.A. was found to be enhanced by the simultaneous administration of NaCl, and in the rat, at least, by partial nephrectomy. Similar lesions were seen in rats long exposed to non-specific damaging agents, particularly cold. This was tentatively interpreted as being due to the endogenous over-production of salt-active corticoids (5). Since the production of corticoids is under the regulating influence of the anterior pituitary, an investigation was made of the rôle of the hypophysis in the production of lesions characteristic of D.C.A. overdosage (6). It was found that crude anterior pituitary preparations preferentially stimulated kidney growth in normal rats drinking water. This enlargement was not accompanied by any detectable degenerative lesions and has been called the renotrophic effect of the anterior pituitary. However, these same pituitary preparations produced marked nephrosclerosis with extensive cast formation in the renal tubules and marked cardiac hypertrophy in rats sensitized to D.C.A. overdosage by partial nephrectomy and salt treatment. At the present time it is not possible to say how the renotrophic and nephrosclerotic activities are related. They may be two manifestations of the same hormonal stimulus. In nephrosclerosis elicited by anterior pituitary treatment the kidney is always markedly enlarged before nephrosclerotic changes appear. On the other hand, kidney enlargement in rats with a normal salt intake does not terminate with nephrosclerosis even after several months' treatment. The relation between these effects can be clarified only by isolation of the active principles and their comparison in a purified state.

A method for the bioassay of the renotrophic activity of anterior pituitary preparations has been reported (7). The present communication describes a method suitable for the bioassay of the nephrosclerotic activity. Although nephrosclerosis of pituitary origin is accompanied by marked adrenal enlargement, and does not occur in the absence of these glands (8), neither its incidence nor severity is closely correlated with the degree of adrenal enlargement. Moreover, pure adrenocorticotrophic hormone (A.C.T.H.¹) injected at a dose level of 0.1 mgm. per day for 24 days, caused no trace of nephrosclerosis in two immature partially nephrectomized, salt-treated rats. However, adrenal cortical stimula-

¹ Kindly supplied by Dr. C. H. Li, Berkeley, California.

tion was not nearly as marked in these rats as in rats treated with crude anterior pituitary. Until more is known of intermediary changes involved, the nephrosclerotic activity must be measured by direct observation of characteristic lesions.

EXPERIMENTAL. Since the production of D.C.A. elicited nephrosclerosis was inhibited by the administration of methyl testosterone (9), we employed male

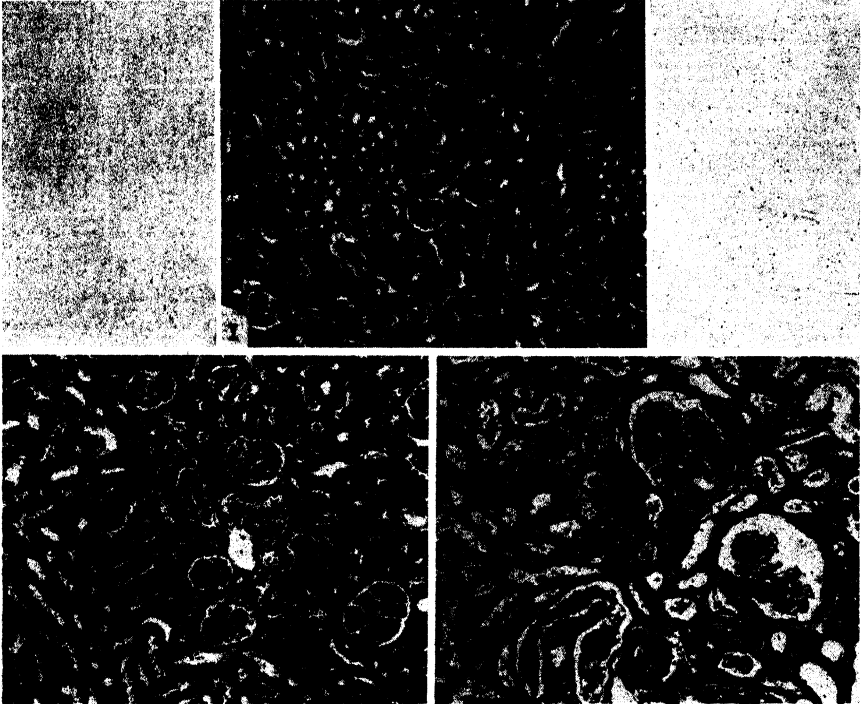


Fig. 1 (Top center). Section of a kidney of a rat of experiment II, group 1, showing normal glomeruli, tubules and arterioles.

Fig. 2 (Lower left). Section of a kidney of a rat of experiment II, group 2, showing glomerular enlargement, slight hyalin accumulations in glomerular spaces, and somewhat dilated tubules some of which contain small hyalinized casts. This would be diagnosed microscopically as a + degree of nephrosclerosis. At this stage the arteriolar walls appear relatively normal.

Fig. 3 (Lower right). Section of a kidney of a rat of experiment II, group 4, showing typical extensive glomerular, tubular and arteriolar changes. This would be diagnosed microscopically as a +++ degree of nephrosclerosis.

castrate or female rats. In preliminary experiments no nephrosclerosis was seen after 14 days treatment with anterior pituitary extract, while after six weeks, a very extensive development of renal lesions occurred. Consequently, a treatment period of 28 days was empirically chosen as suitable. We investigated first, the dose of lyophilized anterior pituitary (L.A.P.) necessary to produce a significant response. Six groups of ten female albino rats, weighing 40 to 60

grams, were partially nephrectomized, given 1 per cent NaCl solution to drink and "purina fox chow"² pellets to eat. L.A.P., suspended in 1 per cent NaCl, was injected subcutaneously in two, 0.2 cc. injections daily for 28 days beginning one day postoperatively. Doses ranged from 5 to 30 mgm. per day. The day after the last injection, the rats were bled and the incidence and severity of macroscopic kidney lesions were noted. The kidneys, hearts and adrenals were fixed in Suza, transferred after 24 hours to 4 per cent formalin, weighed on an analytical balance, and sectioned. The severity of nephrosclerosis, both macroscopically and microscopically, was designated by using a scale of 0 to ++++. The criteria employed were macroscopically, the presence of diffuse fine mottling of the decapsulated surface, and microscopically arteriolonecrosis, capillary tuft hyalinization, and cast formation with resultant tubule dilatation and atrophy (fig. 1 to 3).

The results are tabulated in table 1, experiment I. The number of rats indicated in each group are the numbers surviving until autopsy. The doses of L.A.P. employed are expressed in milligrams injected daily. Shown also are the average initial and final body weights, the adrenal weights in milligrams, the heart weights in grams per 100 grams body weight, and kidney weights in milligrams per 100 square centimeters body surface area. The incidence and severity of nephrosclerosis diagnosed microscopically are expressed as a percent of the maximum possible. Kidney weights are expressed in terms of body surface area rather than body weight because, as MacKay and MacKay (10) have shown, the correlation with the former is much better than with the latter. We used the constant 9.1, suggested by Benedict (11) in the formula $S = K \times W^{2/3}$, where S is the surface area in square centimetres, K , the constant, W , the weight of the rat in grams. Only the incidence and severity of nephrosclerosis diagnosed microscopically is given since after a little experience that diagnosed macroscopically is almost identical. Microscopic diagnoses are preferred because they can be rechecked.

From the table it will be seen that a daily dose of 5 mgm. of L.A.P. induced a marked somatotrophic response, increased adrenal and relative heart and kidney size, but caused no detectable kidney damage. As the dose was increased, the somatotrophic response increased also. The adrenal and relative heart size did not increase regularly; nor were they closely correlated with the increase in the incidence or severity of kidney lesions. For example, there was no statistically significant difference between the cardiotropic effect of 5 and 20 mgm. of L.A.P. The relative increase in kidney size paralleled rather closely the increase in incidence and severity of nephrosclerosis. There was a gradual decrease in the strength of the correlation between kidney size and body surface area, as shown by the marked increase in standard errors. It appears as though the normal mechanism which tends to control this relation breaks down. From this experiment it is clear that a daily dose of 20 to 30 mgm. of L.A.P. elicits an adequate response in 28 days.

² A commercial food prepared by Ralston Purina Co. Ltd., Montreal.

Since female albino rats were less available than male hooded rats, the same assay method was tested with hooded male castrates, weighing 40 to 60 grams. Doses of 20, 25 and 30 mgm. were administered daily. Results are shown in table 1, experiment II. The nephrosclerotic effects were entirely comparable, although considerable differences were observed in the organ sizes of the two types of test animals with and without L.A.P. treatment.

TABLE 1
Nephrosclerotic activity of lyophilized anterior pituitary

EXPERIMENT NO.	GROUP NO.	DOSE OF L.A.P. mgm./day	NO. OF RATS	BODY WEIGHT		ORGAN WEIGHTS			NEPHROSCLEROSIS	
				Initial	Final	Adrenal mgm. \pm S.E.	Heart* %/B.W.	Kidney, mgm./100 sq. cm. S	% incidence	% severity
I	1	0	8	53	123	14 \pm 0.7	0.31 \pm 0.01	353 \pm 11	0	0
	2	5	9	51	158	23 \pm 1.4	0.41 \pm 0.02	411 \pm 10	0	0
	3	10	9	52	175	32 \pm 1.1	0.41 \pm 0.02	434 \pm 17	11	4
	4	15	9	50	182	38 \pm 2.3	0.44 \pm 0.02	482 \pm 22	22	7
	5	20	7	50	191	37 \pm 2.7	0.44 \pm 0.02	524 \pm 38	86	43
	6	30	5	55	171	102 \pm 6.6	0.60 \pm 0.04	708 \pm 68	100	83
II	1	0	10	51	104	27 \pm 1.8	0.40 \pm 0.02	417 \pm 12	0	0
	2	20	8	51	131	64 \pm 2.1	0.46 \pm 0.03	704 \pm 32	100	46
	3	25	5	50	179	80 \pm 1.4	0.50 \pm 0.03	929 \pm 52	100	60
	4	30	10	50	155	72 \pm 3.7	0.52 \pm 0.03	925 \pm 86	100	88
III	1	30	6	49	164	72 \pm 6.6	0.47 \pm 0.03	937 \pm 77	100	81
	2	30	6	49	126	72 \pm 6.9	0.54 \pm 0.03	946 \pm 80	71	52
	3	0	9	48	132	33 \pm 1.6	0.33 \pm 0.02	428 \pm 10	0	0
IV	1	30	9	49	115	72 \pm 4.5	0.49 \pm 0.03	686 \pm 39	89	47
	2	30	7	49	154	74 \pm 4.2	0.48 \pm 0.03	773 \pm 27	100	81
	3	30	5	49	163	89 \pm 4.6	0.51 \pm 0.04	810 \pm 62	100	89

* In experiment I both ventricles and auricles were weighed; in the other experiments, only the ventricles.

All rats received purina fox chow ad libidum.

In experiments I to III the duration of treatment was 28 days.

In experiments IV rats of group 1 were treated 2 weeks, group 2, 3 weeks and group 3, 4 weeks.

In these two experiments, 40 to 60 gram rats were employed because it had been observed in several previous experiments that the incidence of nephrosclerosis was low in large rats given 30 mgm. of L.A.P. per day for 28 days. Severe nephrosclerosis can be produced, however, in large rats if the dose given is increased. For example, when 40 mgm. of L.A.P. were administered twice daily for 28 days to 5, 200 to 240 gram rats suitably sensitized, the incidence and severity of nephrosclerosis induced equaled that seen in 5 similarly sensitized rats one quarter the initial body weight given one quarter the dose.

Preliminary experiments have been made to isolate the principle in the anterior pituitary responsible for the induction of these lesions. Any purification so far attempted, has led to a very great reduction in potency. For example, an alkaline extract of a water suspension of L.A.P. was prepared by adding 0.1 N NaOH to a pH of 10. After standing 10 hours at 0°C., the insoluble material was centrifuged off and the supernatant brought to pH 7 with 0.1 N acetic acid (method similar to that of Burn and Ling (12)). This suspension was made 10 per cent alcoholic with absolute ethanol, added very slowly with stirring. It was prepared weekly, stored at 0°C., and tested in 10, 40 to 60 gram female albino rats at a dose level equivalent to 30 mgm. of L.A.P. given in two injections daily. The L.A.P. used to make the extract was the same batch as was employed in experiment I and the test was carried out at the same time as group 6, experiment I. Although final weights were not significantly different, the incidence and severity of nephrosclerosis was reduced from 100 per cent and 83 per cent respectively in the L.A.P. treated group, to 67 percent and 28 percent in the extract treated group. Another alkaline extract of L.A.P. (pH 8)³ was brought to pH 6 and half saturation with ammonium sulphate. The precipitate was centrifuged off, brought to pH 8 to bring it into solution, and dialyzed until the dialysate was free of suphate. The suspension obtained was brought to pH 7.5 and tested in castrate male hooded rats sensitized in the usual manner. The material induced a somatotrophic response but no trace of nephrosclerosis after 28 days' treatment. This loss of activity is not due to lability in alkaline solutions as shown by the following experiment. L.A.P., suspended in water, was brought to pH 10 with 0.1 N NaOH, and stored in the refrigerator 10 hours. The pH was then brought to 7.0 with 0.1 N acetic acid and the volume adjusted such that the equivalent of 25 mgm. of L.A.P. was administered daily in two 0.3 cc. injections. The nephrosclerotic activity of L.A.P. so treated was compared with that of untreated L.A.P. in two groups of 10 female hooded rats weighing 40 to 60 grams. Both groups were partially nephrectomized and given 1 percent NaCl to drink. After 28 days treatment, the incidence and severity of nephrosclerosis with the alkali-treated L.A.P. were 75 per cent and 54 per cent, and with the untreated L.A.P., 100 per cent and 50 per cent respectively. Thus the activity of the L.A.P. was not materially changed by alkali treatment.

When crude L.A.P. is injected subcutaneously, abscesses and skin exulcerations tend to develop. This may have been due in part to the fact that the suspensions were not sterilized. The reactions were considerably less severe when somewhat purified preparations were tested. The nephrosclerotic activity is not due, however, to the injection of an abscess-forming substance, since when an equivalent dose of lyophilized liver is administered, similar skin lesions appear without renal enlargement or nephrosclerosis. This fact was demonstrated with two groups of 10 hooded male castrate rats, weighing 40 to 60 grams, and sensitized to nephrosclerosis in the usual manner. The animals of one group were injected with 15 mgm. of lyophilized beef liver suspended in 0.4 cc. 1 per cent NaCl twice

³ Kindly supplied by Dr. H. Jensen, DesBergers Bismol Laboratories, Montreal.

daily, and those of the second, with the same amount of similarly suspended L.A.P. After 28 days' treatment, the skin lesions in both groups were similar, but the incidence of nephrosclerosis in the first group was zero and in the second, 100 per cent.

In all the experiments described above, the animals received "purina fox chow" *ad libitum*. Since the anterior pituitary treated animals ate much more than untreated controls, it was thought that overeating might be a contributory factor in the development of these kidney lesions. To investigate this, the following experiment was performed. Three groups of ten male castrate hooded rats were sensitized by partial nephrectomy and the administration of 1 per cent NaCl to drink. The animals of groups 1 and 2 were injected subcutaneously with 15 mgm. of L.A.P.; suspended in 0.3 cc. 10 per cent alcohol, twice a day for 28 days. Those of group 3 were injected similarly with an equivalent dose of casein. The rats of the first and third groups received "purina fox chow" freely, while those of the second were pair fed with those of the third. The average daily food intake per rat during the period of treatment was 17 grams in the first group, and 13 grams in the second and third. Unfortunately the daily fluid intake was not measured. Results are tabulated in table 1, experiment III. The incidence and severity of nephrosclerosis appeared to be slightly reduced but the adrenal and proportional heart and kidney weights were not influenced by food restriction. Nephrosclerosis was not prevented by maintaining the food intake of treated rats at the level of the untreated.

In a number of assays, animals treated daily with 20 to 30 mgm. of L.A.P. died with severe nephrosclerosis before twenty-eight days had elapsed. To investigate whether the length of treatment could be reduced, groups of 10 male castrate hooded rats were sensitized as usual, and injected daily with 30 mgm. of L.A.P. suspended in 10 per cent alcohol for two, three and four weeks. Results are summarized in table 1, experiment IV.

The incidence and severity of nephrosclerosis was no greater after four than after three weeks' treatment. The proportional heart and kidney size increased steadily with the duration of treatment, but the adrenal size was not significantly greater after four than after two weeks. This assay method has been found very satisfactory for use in our studies on the effect of the diet on L.A.P. induced nephrosclerosis (unpublished).

SUMMARY

1. A method has been developed for assaying the nephrosclerotic activity of anterior pituitary preparations. In this method, female or male castrate, albino or hooded rats may be employed. They should weigh 40 to 60 grams initially, and should be sensitized by partial nephrectomy and the administration of 1 per cent NaCl to drink. In such animals, the subcutaneous injection of 10 to 15 mgm. of lyophilized anterior pituitary (L.A.P.) in water, saline or 10 per cent alcoholic suspension, twice daily for 21 days, consistently induces an incidence of nephrosclerosis of 80 to 100 per cent with an average severity of 50 to 90 per cent of the maximal. This is as high an incidence and severity as is seen after 42

days' treatment with one flat 50 mgm. desoxycorticosterone acetate pellet implanted subcutaneously in similarly sensitized female albino rats (13).

2. Equally severe lesions can be produced in adult rats, if the dose is increased.

3. Preliminary efforts to isolate the active pituitary principle have not been successful as yet, due to a rapid loss of activity with even slight purification. The activity is not impaired by treatment with NaOH at pH 10 for 10 hours at 0°C..

4. The pituitary preparations employed to produce these lesions invariably caused local skin damage, but beef liver produced similar skin damage with no nephrosclerosis.

5. L.A.P. produced nephrosclerosis even in rats in which overeating was prevented.

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REFERENCES

- (1) SELYE, H. *Canad. M. A. J.* **47**: 515, 1942.
- (2) SELYE, H. AND H. STONE. *Proc. Soc. Exper. Biol. and Med.* **52**: 190, 1943.
- (3) SELYE, H. AND C. E. HALL. *Arch. Pathol.* **36**: 19, 1943.
- (4) SELYE, H., C. E. HALL AND E. M. ROWLEY. *Canad. M. A. J.* **49**: 88, 1943.
- (5) SELYE, H. *Rev. Canad. de Biol.* **2**: 501, 1943.
- (6) SELYE, H. *Canad. M. A. J.* **50**: 426, 1944.
- (7) HAY, E. C. *In press.*
- (8) HALL, C. E. AND OTHERS. *To be published.*
- (9) SELYE, H. AND E. M. ROWLEY. *J. Urol.* **51**: 439, 1944.
- (10) MACKAY, L. L. AND E. M. MACKAY. *This Journal* **83**: 191, 1927.
- (11) BENEDICT, F. G. *Ergebn. d. Physiol.* **36**: 300, 1934.
- (12) BURN, J. H. AND H. W. LING. *Quart. J. Pharmacy* **6**: 31, 1933.
- (13) SELYE, H., J. MINTZBERG AND E. M. ROWLEY. *J. Pharmacol. and Exper. Therap.* **85**: 42, 1945.

MOVEMENTS OF BODY WATER IN RESPONSE TO ACUTE BLOOD LOSS

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The anemia which follows blood loss actually represents a re-expansion of the plasma volume without replacement of the erythrocytes. In the untreated post-absorptive subject the water and the electrolytes of the interstitial space and of the cells are the only possible sources of this fluid. It has been stated, on the basis of indirect evidence, that both these phases contribute to the restoration of the plasma volume (1, 2). This point of view appeared to have been confirmed experimentally by Stewart and Rourke in 1936 (3). These investigators concluded from potassium excretion studies that cell water was transferred to the extracellular space and to the plasma following blood loss. In 1942 Lands and Johnson reported an increase in the "sulfocyanate available water" after hemorrhage (4). This was interpreted as further evidence of a movement of water from cells into the tissue spaces. Ashworth and Kregel stated in the same year, however, that measurements made with sulfocyanate indicated that cell water increased after hemorrhage at the expense of extracellular water (5). The evidence for transfers of body water following blood loss is, therefore, contradictory.

It is now known that starvation *per se* is accompanied by a loss of cell potassium (6). It is possible, therefore, that the findings of Stewart and Rourke represent the effect of starvation rather than of hemorrhage, since their dogs were fasted. Moreover, sulfocyanate has been shown to possess distinct limitations as a measure of extracellular water (7). The question of fluid transfer following acute blood loss has, therefore, been investigated in non-fasting dogs, using the chloride balance to measure changes in the extracellular fluid volume. It has been found that cell water does not contribute to this re-expansion of the plasma volume.

PROCEDURE. Two hundred and fifty cubic centimeters or more of blood were withdrawn rapidly by jugular vein puncture from post-absorptive unanesthetized female dogs weighing 7 to 14 kgm. The effect of this procedure upon the movement of body fluids during the next 10 to 80 minutes was observed in 6 experiments (nos. 1 to 6, table 1). In 2 other experiments the removal of blood was repeated until fatal exsanguination occurred at 132 and 192 minutes (nos. 7 and 8, table 1). In these 2 experiments and in the previous 6 food and water were withheld.

Observations over a prolonged period were made in 4 other experiments. Two lasted approximately 24 hours. In these food was withheld but free access to water was allowed (expts. 5 and 6, table 2). Two other animals were studied for 48 hours. A proprietary dog food of known composition was given in addi-

tion to water (expts. 9 and 10, table 2). In all of these 4 studies the major loss of blood occurred at the start of the experiment during an interval of several minutes. Subsequently blood was withdrawn only for analysis.

METHODS. Alterations in the volume of extracellular fluid were calculated from changes in the serum concentration and the net balance of chloride according to the usual procedure (8).

The changes in the volume of cell water were obtained by subtracting the change in extracellular water from the change in total body water. The total

TABLE 1
Acute experiments: balance data and blood analyses

EXPERIMENT	TIME*	WEIGHT	BLOOD WITH- DRAWN	FECES	BALANCE OF Cl	WHOLE BLOOD		SERUM		SERUM	WATER	NET BALANCE OF WATER†		
						NPN	Hema- tocrit	Total protein	H ₂ O	Cl	K	ΔW	ΔE	ΔI
	min.	kgm.	cc.	grams	m.Eq.	mgm. per cent	per cent cells	grams per cent	grams per cent	m.Eq. per liter	m.Eq. per liter	liters	liters	liters
1	0	7.93				47	34.8	6.01	93.5	113.8	5.81			
	75	7.62	292	14	-28		33.7	4.85	95.0	119.2	5.22	-0.02	-0.05	+0.03
2	0	8.83				40	38.1	6.31	93.7	115.8	5.53			
	65	8.39	330	50	-35		29.8	4.88	94.9	117.8	5.58	-0.08	-0.05	-0.03
3	0	8.21				23	43.2	7.02	92.7	117.7	5.93			
	64	7.73	250	78	-23		35.5	5.95	93.6	116.3	4.87	-0.16	±0	-0.16
4	0	8.81				35	44.8	5.14	94.7	116.6	3.87			
	63	8.50	310	0	-28		32.0	3.82	95.9	118.5	3.24	-0.01	-0.04	+0.03
5	0	8.71				29	32.6	5.76	93.9	114.7	5.59			
	80	8.22	295	170	-28		23.7	4.41	95.5	113.6	4.62	-0.06	+0.02	-0.08
6	0	14.74				30	44.6	5.79	94.1	118.0	4.64			
	10	14.37	365	0	-32		39.4	4.95	94.8	121.8	4.43	-0.07	-0.10	+0.03
7	0	12.77				24	52.1	6.33	93.4	116.4	5.00			
	65	12.20	430	80	-28		45.5	5.38	94.5	120.0	4.07	-0.08	-0.10	+0.02
Mult. hemorr.	132	12.01	144	0	-14	43	45.1	5.56		120.0	5.74	-0.05	±0	-0.05
8	0	7.92				28	35.9	6.00		112.3	5.27			
	192	7.36	414	75	-29		25.3	4.32		114.8	4.67	-0.09	-0.01	-0.08
Mult. hemorr.														

* Time is from start of hemorrhage. Balance data are non-cumulative, and are for period ending at time given.

† Net change in total water (ΔW), extracellular fluid (ΔE), and intracellular fluid (ΔI), i.e., after subtracting the water lost in hemorrhage.

water balance was derived from the change in body weight corrected for water and solids ingested, excreted, or removed in the blood sample, and, when necessary, for food metabolized (8). In short experiments the water content of serum was measured directly as the difference between wet and dry weights. In prolonged experiments the water of serum was calculated from the total protein content by the formula: Water of serum = $99.30 - (0.889 \text{ protein of serum})$ (9). The serum content of the blood was obtained from the relative cell volume of whole blood defibrinated anaerobically with mercury (10, 11). The concentration of serum potassium was measured in all but one experiment (12).

RESULTS. There is no evidence from chloride balance studies in acute experiments or in the 2 studies with fatal exsanguination that extracellular fluid and plasma were replenished by movement of water from cells (table 1). At a time when the hematocrit had dropped markedly the extracellular fluid volume remained constant, or actually declined. The cell water decreased significantly (more than 0.05 liter) in only 3 of the 8 experiments. The concentration of serum potassium remained unaltered.

TABLE 2

Prolonged experiments with water and with and without food: balance data and blood analyses

EXPERIMENT	TIME	WEIGHT	INTAKE		OUTPUT					BAL- ANCE	WHOLE BLOOD		SERUM protein	SERUM WATER		ΔW^{**}	ΔE	ΔI
			Food†	H ₂ O	Blood	Urine vol.	Urine N	Feces	Cl		NPN	Hemato- crit		Cl	K			
	hrs.	kgm.	grams	cc.	cc.	cc.	grams	grams	m. Eq.		mgm. per cent	per cent cells	grams per cent	m. Eq. per liter	m. Eq. per liter	liters	liters	liters
5* H ₂ O ad lib	5.3	8.32	0	280	70	20		30	-6				4.69	111.0		+0.21	+0.04	+0.67
	24.5	7.61	0	60	15	460	2.52	30	-11		23		5.53	118.0		-0.66	-0.20	-0.46
6* H ₂ O ad lib	4.5	13.96	0	0	85	15		190	-11			32.3	4.32	118.2	4.71	-0.15	+0.09	-0.24
	10.5	13.97	0	275	100	225		0	-18					114.6		+0.09	+0.02	+0.07
	23.0	13.74	0	50	40	40		0	-3		25		4.60	113.6		-0.15	+0.03	-0.18
9 H ₂ O and food	0	8.28									35	35.0	5.58	111.6	4.53			
	4.5	7.74	0	0	280	23		160	-20			30.5	5.31	114.0	5.02	-0.12	-0.03	-0.09
	11.0	7.84	0	300	15	72		34	-1				5.23	109.8		+0.15	+0.06	+0.09
	24	8.09	200	500	20	190	5.80	10	+52		44	15.9	4.96	119.8	4.57	+0.14	+0.27	-0.13
	48	8.40	200	1000	50	480	7.92	270	+20		36		5.19	112.7	4.61	+0.47	+0.70	-0.23
10 H ₂ O and food	0	8.10									25	39.0	6.60	114.0	5.28			
	3.5	7.71	0	0	265	18		0	-18			30.9	4.90	114.4	4.83	-0.10	±0	-0.10
	7.5	7.79	0	125	15	30		0	-1				5.58	121.5	5.54	+0.09	-0.12	+0.21
	24	7.97	200	880	15	615	6.88	0	+49		24	22.6	5.39	114.1	4.33	+0.19	+0.53	-0.34
	48	8.14	200	870	50	680	8.37	130	+13		34		5.85	117.3	5.08	+0.21	+0.08	+0.13

* Data for initial periods are given in table 1.

† Consisted of 311 m. Eq. Cl and 73.5 grams N per kilogram dried food. Time is from start of hemorrhage. Balance data are non-cumulative, and are for period ending at time given.

** Water of oxidation not included in calculation of water balance during periods shorter than 12 hours, or with urine volumes smaller than 0.05 liter.

In the studies prolonged to 23 and 24.5 hours (expts. 5 and 6 in table 2) in which water was offered without food only small amounts were taken. The animals were in negative water balance at the end of the experiment. This water was lost predominantly or entirely from the cells. In experiment 5 the extracellular volume decreased, while in experiment 6 it expanded.

In the 48 hour experiments (nos. 9 and 10 in table 2) the animals drank freely and ate large amounts of food. As a result the total balance of body water was positive, and the extracellular fluid volume was definitely expanded at 24 hours. It increased further during the 24 to 48 hour period.

DISCUSSION. In the first hour following acute blood loss the serum protein

concentration and the relative cell volume drop sharply. The changes indicate that the plasma volume has re-expanded. During this same period it is usually not possible to detect any significant alteration in the volumes of either the extracellular or the intracellular fluid. If, under these conditions, the chloride space is a valid measure of changes in extracellular volume, plasma must have been diluted by interstitial fluid alone. The most important source of possible error lies in the assumption that cellular chloride does not change significantly following hemorrhage. The chloride of the erythrocytes, the largest store of tissue chloride in the body, appears to be stable under these conditions (13). The chloride in the other tissues of the body is quantitatively unimportant insofar as calculation of changes in cell water are concerned. Even if all the chloride present in cells other than the erythrocytes were transferred, the measurement of extracellular fluid volume by means of the chloride space would not be significantly affected.

Our results differ from those of Lands and Johnson (4) who found interstitial fluid expanded 1 to 2.5 hours after blood loss. The experimental conditions were not, however, comparable, since they used anesthetized cats and measured extracellular water by the sulfocyanate space. We have also been unable to confirm the findings of Ashworth and Kregel (5) who inferred, from measurement by the sulfocyanate method, that anesthetized dogs had an increase in cell water at some unspecified time after hemorrhage.

In experiments prolonged over a period of a day or two the interpretation of fluid movements is complicated by the additional factors of dehydration and starvation. In dogs deprived of water the negative water balance is shared in variable proportions by both cellular and extracellular phases (9). Loss of cell water is always present with starvation (9). A decrease in the volume of intracellular fluid of an animal maintained without food or water following a large hemorrhage can not, therefore, be interpreted as replenishment of extracellular water at the expense of cell water. Stewart and Rourke (3) bled animals after 4 to 5 days without food but with water. The hemorrhage was followed by an increased excretion of potassium in excess of nitrogen and pointed to a loss of cell fluid. These findings do not, however, necessarily pertain to movements of water following hemorrhage in animals not previously starved. The stability of the concentration of serum potassium in our experiments while the dogs were anuric indicates that cell water is not transferred shortly after hemorrhage. This provides evidence independent of the chloride space calculations that cell water has not been transferred to the extracellular spaces.

In the experiments prolonged to 23 and 24.5 hours the animals were in negative water balance because of insufficient intake. The decrease in body water was predominantly intracellular. Although part of this loss may indicate movement of water into the extracellular space following hemorrhage, it is probable that in the main it represents the effect of dehydration and starvation. In the last 3 periods of the 48 hour experiments with free intake of food and water the cell water varied in amount. This was not apparent, however, as a trend, and suggests adjustment to the intake of water and electrolytes rather than to hemor-

rhage. With adequate intake during recovery from hemorrhage the depleted extracellular fluid is evidently replenished from exogenous sources rather than by movements of water from cells.

CONCLUSIONS

1. Re-expansion of plasma volume shortly after acute blood loss is accomplished by a transfer of interstitial fluid only.
2. The extracellular fluid is subsequently replenished from exogenous sources, provided that the intake of food and water is adequate.

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REFERENCES

- (1) MILROY, T. H. *J. Physiol.* **51**: 259, 1917.
- (2) KERR, S. E. *J. Biol. Chem.* **67**: 689, 1926.
- (3) STEWART, J. D. AND G. M. ROURKE. *J. Clin. Investigation* **15**: 697, 1936.
- (4) LANDS, A. M. AND W. JOHNSON. *Proc. Soc. Exper. Biol. and Med.* **49**: 123, 1942.
- (5) ASHWORTH, C. T. AND L. A. KREGEL. *Arch. Surg.* **44**: 829, 1942.
- (6) WINKLER, A. W., T. S. DANOWSKI, J. R. ELKINTON AND J. P. PETERS. *J. Clin. Investigation* **23**: 807, 1944.
- (7) ELKINTON, J. R. AND M. TAFFEL. *This Journal* **138**: 126, 1942.
- (8) ELKINTON, J. R. AND A. W. WINKLER. *J. Clin. Investigation* **23**: 93, 1944.
- (9) DANOWSKI, T. S., J. R. ELKINTON AND A. W. WINKLER. *J. Clin. Investigation* **23**: 816, 1944.
- (10) EISENMAN, A. J., L. B. MACKENZIE AND J. P. PETERS. *J. Biol. Chem.* **116**: 33, 1936.
- (11) EISENMAN, A. J. *J. Biol. Chem.* **71**: 607, 1927.
- (12) HALD, P. M. *J. Biol. Chem.* **103**: 471, 1933.
- (13) DANOWSKI, T. S., A. J. EISENMAN, J. R. ELKINTON AND A. W. WINKLER. Unpublished data.

A STUDY ON THE PATHWAY FROM THE MEDIAL GENICULATE BODY TO THE ACOUSTIC CORTEX IN THE DOG¹

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It is known that the two ears are necessary for the perception of the direction of a sound source. The sensations produced have been referred to as binaural effects and depend on the central nervous system. Several factors are present when the sounds are complex, but the ability to locate the direction of a source exists with the use of pure tones. Generally, for pure tones the ability to localize the direction is attributed to the physical factors of phase and intensity differences at the two ears (5, 14, 15, 16). With brief sound pulses, the time difference of arrival of the sound at the two ears has been considered also as a factor by some investigators (6), although this has certain properties in common with phase. When tones of equal intensities and equal frequencies below about 1000 dv. are led to the two ears separately and the phase varied, there occurs an angular displacement of the sound image toward the side of the leading phase (14, 16). The angular displacement from the midline has been shown to be a relatively constant function of the phase angle (16). Within the range of frequencies between 2000 and 4000 dv. the ability to localize the direction diminishes to a minimum (15) and rises again to a maximum above 4000 dv. In this upper range phase shift does not cause displacement, but intensity differences at the two ears cause movement of the sound image to the side of the greater intensity.

Since localization by phase differences at the two ears is a function of time, then it would appear that a circuit in the nervous system capable of handling this time function is present. That the circuit is simple is suggested by the fact that the change from phase to intensity for localization occurs at a frequency that is about equal to the maximum repetition rate for nerve fibers of the central nervous system. A second assumption is that the two cerebral hemispheres are necessary for the perception of the direction of a sound source.

Kemp, Coppee and Robinson (7, 8) have failed to find definite interaction in the nerve fibers of the lateral lemniscus to impulsive stimulation of both ears. It is the purpose of this report to present evidence for the existence of a neurone common to both ears and reaching the cerebral cortex of all three acoustic areas in the dog (18).

METHODS. The present investigation was performed on fifteen dogs, anes-

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thetized with pentobarbital sodium. The cerebral cortex was exposed on one side (the left was used in all experiments) and the brain was surrounded with cotton soaked in Ringer's solution. A grounded lead ring was embedded within the moist cotton. Oscillograms of the cortical responses (voltage deflections) were taken from a pair of electrodes, one situated upon the cotton surrounding the brain, which served as the indifferent electrode, and the other was mounted in a holder which was moved over the surface of the brain in 2 mm. co-ordinate divisions. Both electrodes consisted of small cotton tufts tied to the ends of silver wires and moistened with Ringer's solution. These electrodes were led into the grids of a five-stage differentially-balanced push-pull amplifier, whose output was fed into the deflecting plates of a five-inch cathode-ray oscillograph.

Each cochlea was uncovered by dissecting from the ventral side between the mandible and trachea into the auditory bulla. The bone of the dorsal wall of the bulla was chipped away with a dental chisel to gain access to the inner ear, where the bony spiral appeared as three ridges within the chambers of the cochlea. In several experiments a drill hole was made into a specific part of the inner ear, without chipping away the wall. Stimulation of a local group of nerve fibers was performed through a pair of electrodes consisting of stainless steel wires twisted together and insulated from each other by means of Glyptal varnish baked on the surface. The wires, bared by cutting off the tips, were placed at any desired location on the spiral lamina of the exposed cochlea, or inserted through the drill holes. The electrical stimuli led through these wires consisted of condenser discharges, synchronized with the sweep circuit of the cathode-ray oscillograph.

To determine the effect of a precedent response upon another, two electrical stimuli separated by any time interval were sent: 1, through one pair of wires to a location on the bony spiral; 2, through separate pairs of wires to two locations in the same cochlea, and 3, to different or symmetrical positions in opposite ears. The wires were placed on symmetrical positions in opposite ears by direct observation and the cortical areas were studied by means of the co-ordinate electrode carrier. Threshold cortical responses were used in most experiments, but cortical responses from one point were made maximum by means of strong stimuli in some experiments. When two successive stimuli to symmetrical parts of opposite ears were used, the stimuli were adjusted to make the responses from a single point on the cortex equal or nearly so for the two sides.

The responses of the radiations from the medial geniculate body to the cortex, and those in the region of the medial geniculate body were studied by means of needle electrodes made of sewing needles whose tips had been ground to fine points and insulated with Glyptal varnish. The needles were then baked after which the insulation on the tips was removed for a distance of about 0.5 mm. by grinding on a fine stone. Two needles spaced 2 mm. apart were thrust in steps of fractions of a millimeter through the cortex into the white matter of the radiations until responses were encountered. If no responses were found the needles were withdrawn and reinserted 2 mm. away, and the procedure repeated until responses were found. Depths of the thrusts were recorded and points of

responses were noted. Subsequent preparation of the brain by gross section and in some instances by frozen section showed the needle tracts which were then measured to locate the site of the responses. Because of the large size of the bare tips of the needle electrodes, it was considered that only summed records from relatively numerous elements would be obtained rather than from a single neurone.

RESULTS. The responsive areas in one cerebral hemisphere resulting from an electrical stimulus applied to a localized portion of one bony spiral coincided within the error of observation with the responsive areas in the same hemisphere due to a similar localized electrical stimulus applied to the symmetrical point in the opposite cochlea. Figure 1 illustrates the responses of the dorsal and ventral acoustic areas to strong electrical stimuli delivered through drill holes in the apex

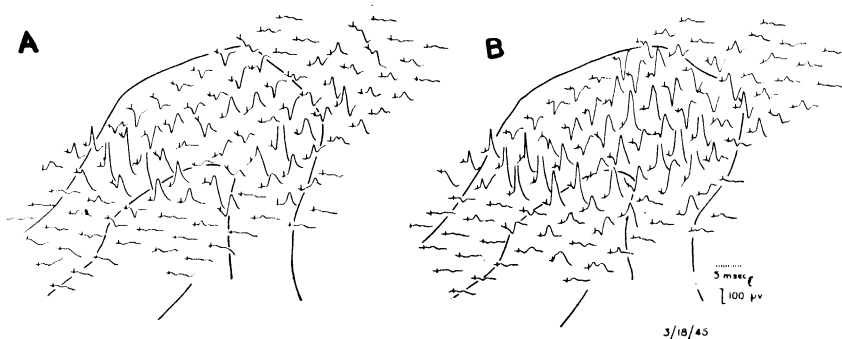


Fig. 1. Oscillograms of responses in left cerebral cortex to electrical stimuli applied through holes drilled into the apex of each cochlea. A: responses from left ear. B: responses from right ear. Stimulation of the middle turn seems apparent from the wide distribution of the responses in particular toward the basal region. The third area was not studied in this experiment. Two millimeter co-ordinate divisions. Upward deflection is surface positive.

of each cochlea. The extensive spread of the responses indicates that the middle turn of the cochlea was also stimulated. In figure 3 responses are shown in the third area to symmetrical stimulation of the basal turn of each cochlea. The same coincidence was found for stimulation of any symmetrical parts of each cochlea for all three acoustic areas.

Comparison of the individual responses at each cortical point to stimulation of symmetrical points in opposite ears showed similarity of latency, duration, wave form, and initial sign (figs. 1 and 3). The latencies of the responses in all areas varied from 7–10 msec., but the latencies of responses at a single cortical point for both ipsi-lateral and contra-lateral stimulation agreed within 1 msec. The initial sign was generally positive, particularly for threshold stimulation, but with strong stimuli regions of initial negativity were scattered throughout the areas (fig. 1). The changes in initial sign from negative to positive occurred at the same contours for stimulation of the two sides. Amplitude of responses to contra-lateral stimulation in general appeared to be greater than those of the

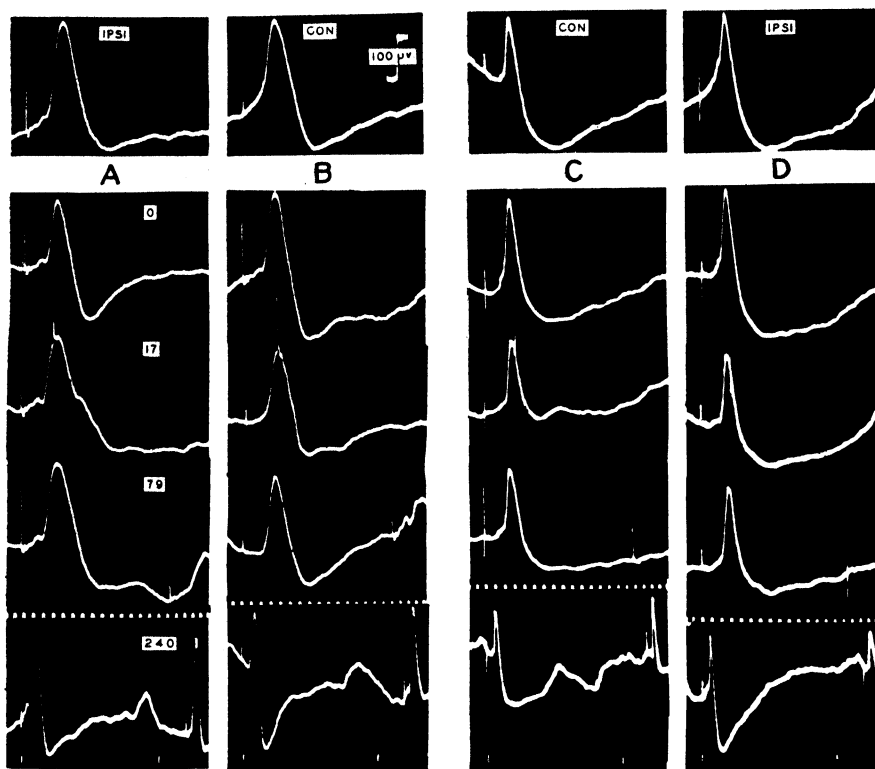


Fig. 2. 3/18/45. Top records: responses to electrical stimuli applied to each apex alone. *IPSI*: Ipsi-lateral apex. *CON*: Contra-lateral apex. Columns A and B, dorsal area. Columns C and D, ventral area. Columns A and C: ipsi-lateral stimulus followed by contra-lateral. Columns B and D: contra-lateral followed by ipsi-lateral. Time base for upper four records: 5 msec., and for the bottom record: 200 msec. Numbers in column A give the time in milliseconds between stimuli for all columns. Note beginning of recovery of the response to the second stimulus at 79 msec. Upward deflection: surface positive.

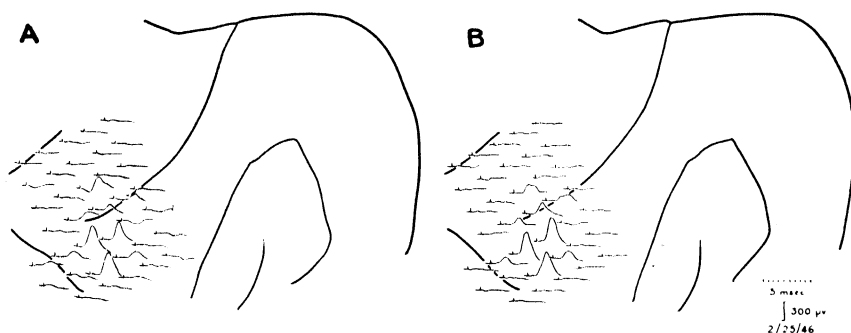


Fig. 3. Comparison of responses in third area of left cerebral cortex to electrical stimuli applied to the base of each cochlea. A: stimulation of left base. B: stimulation of right base. Note relative absence of responses on coronal gyrus. Upward deflection: surface positive.

ipsi-lateral side, although altering the strengths of the stimuli would result in responses of the same magnitude.

Two electrical stimuli of equal strength delivered in succession to the same part of the bony spiral caused the appearance in the cortex of an absolutely unresponsive period to the second stimulus lasting 20 to 100 msec. This was followed by an interval during which the response to the second stimulus gradually increased in size until it reached its former magnitude. This second period lasted from 100 to 250 msec. or longer. In some experiments the second response had recovered only 50 per cent of its original size in 250 msec. This was most frequently observed upon stimulation of the ipsi-lateral side. The intervals of absolute and relative unresponsiveness tended to increase in duration with the depth of the anesthesia. However, in any one experiment, the durations of the unresponsiveness were the same for stimulation of the basal, middle or apical portions of the bony spiral for all three acoustic areas.

Successive stimuli, applied separately to identical points of opposite ears, resulted in an absolutely unresponsive period (20–100 msec.) on the cortex to the second stimulus, which paralleled in duration for an individual experiment the unresponsive period to successive shocks applied to the same ear. The absolutely unresponsive period was followed by an interval during which the response gradually increased in size to its original magnitude. Precedent stimulation of the ipsi-lateral side in some experiments gave a longer lasting period of unresponsiveness than for precedent stimulation of the contra-lateral side. Typical results are illustrated in figure 2. Similar results were found to occur in all three acoustic areas to stimulation of the basal, middle or apical turns.

In the report on the third area (18) it was indicated that responses resulting from the application of electrical stimuli to separate turns of one bony spiral did not show the unresponsive period at one point in the third area. The potentials merely added algebraically as seen in figure 4. Separate stimuli were applied to non-symmetrical parts of opposite ears, and again the phenomenon of addition of the cortical responses was observed in the third area. Neither facilitation nor inhibition seemed to be present. In various combinations the non-identical parts of the opposite ears were stimulated in succession, but in every case only addition of potentials was observed. C and D of figure 4 illustrates the behavior of the potentials when the ipsi-lateral base and contra-lateral apex were stimulated in succession.

Records taken from the radiations by means of needle electrodes possessed the same unresponsive period as shown by the cortical responses (figs. 5 and 6), when one point in the same ear was stimulated in succession or when symmetrical points in opposite ears were stimulated. The latency of the radiation responses was of the order of 4–6 msec. The initial deflection was positive with respect to an unresponsive electrode. Typical records of the wave form, latency and duration are illustrated in figure 6. In some experiments, sharp deflections were noted on the crest of the slow wave, suggesting impulses in individual units some distance from the tips of the electrodes. However, no attempt was made in the present experiments to record from single fibers.

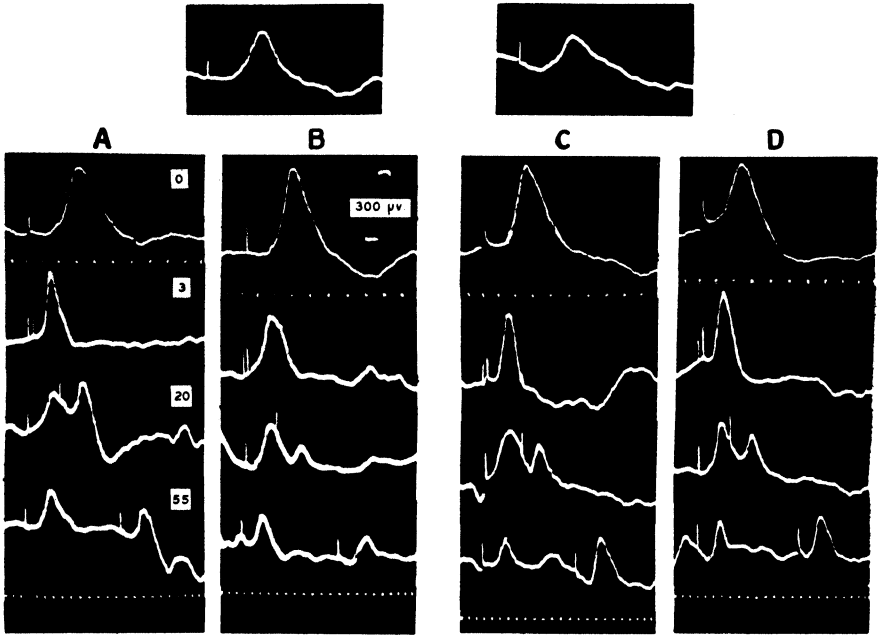


Fig. 4. 2/25/46. Oscillograms from a single point in the third area. Left upper: to stimulation of the ipsi-lateral apex alone. Right upper: to stimulation of the contra-lateral base alone. Responses of ipsi-lateral base and contra-lateral apex are identical, but not shown. Column A: responses to stimulation of ipsi-lateral apex followed by ipsi-lateral base. In column B, the order of stimulation is reversed. Column C: stimulus to ipsi-lateral apex followed by contra-lateral base. Column D: order of stimuli reversed. Numbers in column A give time interval between stimuli. Upward deflection surface positive. Time: 5 msec.

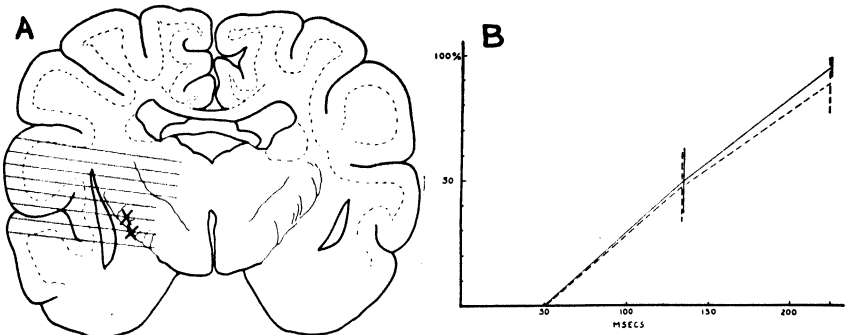


Fig. 5. 4/7/46. A: transverse section of brain showing needle tracts passing through left radiations. Crosses indicate location of impulses obtained by electrical stimuli applied to the apex of each cochlea. Oscillograms of the responses from lower cross are shown in figure 6. B: ordinate indicates per cent recovery in size of the second response. Abscissa gives the time in milliseconds between stimuli. The solid line represents the average of recovery from the radiations using successive stimuli in the combinations of: ipsi-lateral-ipsi-lateral; contra-lateral-contra-lateral; ipsi-lateral-contra-lateral; and contra-lateral-ipsi-lateral. The broken line is the average for the same combinations of stimuli in the dorsal and ventral areas of the same animal.

When the needles were located in the medial geniculate body the same type unresponsive curve was found that was characteristic of the radiation responses, but no long unresponsive period was detected below the medial geniculate body. In several experiments, needles were thrust into the region of the lateral lemniscus as it enters the medial geniculate body. Bipolar needle electrodes oriented in such a manner that both tips were on an isopotential with respect to the

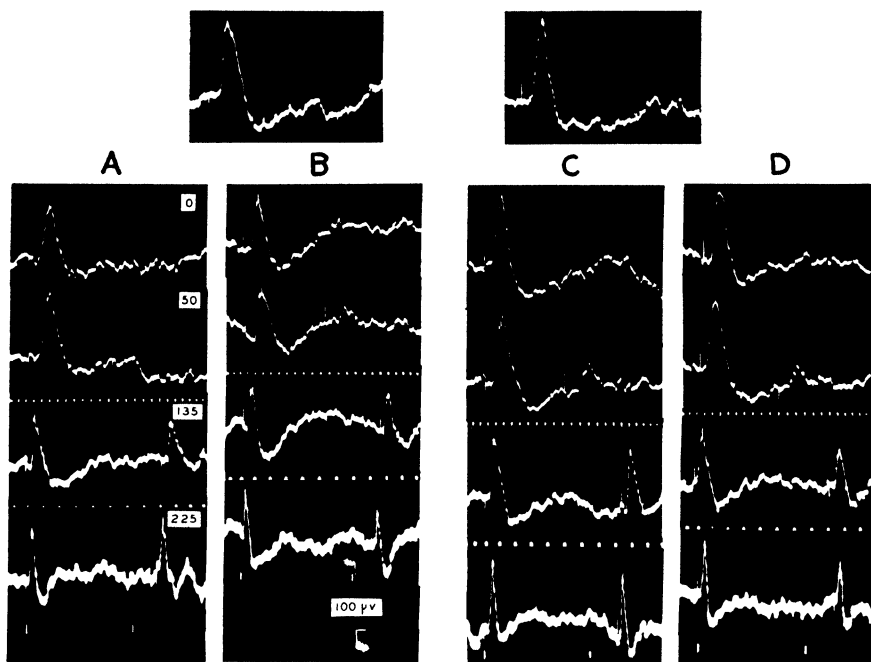


Fig. 6. 3/7/46. Oscillograms of responses from the left radiations. Site of responses shown by lower X in figure 5A. Top left: response to stimulation of left apex alone. Top right: response to stimulation of right apex alone. A: stimulation of left apex in succession with the various time intervals indicated by numbers. B: successive stimulation of right apex. C: stimulation of left apex followed by stimulus to right apex. D: stimulation of right apex followed by stimulus to left apex. Time bases from top to bottom: 5 msec.; 16.8 msec.; and 200 msec. Amplitudes of responses in columns A and B slightly smaller, because these tests were made last.

inferior colliculus, gave potentials whose latencies were approximately 2.3–3.0 msec. Successive stimulation of one point in the same ear, either ipsi-lateral or contra-lateral, resulted in an absolutely unresponsive period of 1 msec. with complete recovery at 2 msec. Successive stimulation of symmetrical points in opposite ears gave results suggesting partial addition of the potentials. No change in latency was observed.

DISCUSSION. An absolutely unresponsive period of 25–30 msec. to successive stimulation of the same spot in the skin has been observed in the cerebral cortex of the cat anesthetized with nembutal by Marshall et al. (11, 12). Cyclic varia-

tions of the optic cortical responses have been described by Bishop and O'Leary (3) and Marshall and Talbot (10). These observations are in agreement with the present findings for successive stimulation of a single group of nerve fibers in the bony spiral of the cochlea. With increased depth of the anesthesia, the unresponsive time at the acoustic cortex lengthened in the same manner as observed in the tactile region of the cortex by Marshall et al. (11, 12). No attempt was made to release the animal from the anesthesia in the present experiments. The radiations showed the same duration of unresponsiveness as seen at the cortical level, but below the medial geniculate body the duration of the unresponsive period was no greater than 1–2 msec. The unresponsive period therefore arises in the medial geniculate body. This is consistent with the findings by Marshall (12) in the tactile system, where only brief unresponsive intervals were found below the nucleus ventralis pars lateralis of the thalamus.

Kemp et al. (7, 8) found the repetition rate in the lateral lemniscus to be of the order of 2500 for pure tones. This appears high if the results of Galambos and Davis (4) for peripheral nerve are considered, and the present findings suggest that repetition in the region of the lateral lemniscus is limited to about 500 and at the most 1000 impulses per second. However, responses in the region of the lateral lemniscus may be actually electric field effects of the inferior colliculus.

The unresponsive period at the acoustic cortex resulting from successive stimulation of symmetrical points on the bony spiral of opposite sides is interpreted as due to the presence of a common neurone arising at the medial geniculate body. No definite conclusion can be made in the present study from the records below the geniculate body. On the other hand, Kemp et al. (7, 8) by employing clicks for stimulation and recording with concentric electrodes concluded that the interaction was absent or very slight in the lateral lemniscus. Talbot and Marshall (17) noted facilitation, but no unresponsiveness to bilateral optic stimulation in the visual cortex.

The possibility that the unresponsive period was due to cortical elements was considered, but the records from the radiations do not support this conclusion. The duration of the unresponsive period to stimulation of symmetrical points on the two sides paralleled the duration for successive stimuli to one point in either ear. Also, in the third area, the addition of potentials due to stimulation of non-identical points in the same or opposite ears is evidence against cortical effects for the phenomenon.

Woolsey and Walzl (19) made the observation in one experiment that stimulation of the extreme end of the basal turn of each cochlea resulted in the identical distribution, latency, wave form and amplitude of responses in the dorsal area of one cerebral hemisphere. The distributions and characteristics of the responses determined in the present experiments for both sides were found to correspond to electrical stimulation of the basal, middle and apical turns of the bony spiral in all three areas of the acoustic cortex in the dog. This represents further evidence that a common neurone arises from the medial geniculate body.

Pavlov (13) has shown with the conditioned salivary reflex, that a dog is una-

ble to make right and left discrimination to a tone when the corpus callosum had been severed previously. Also, it is evident from Allen's work (1) that correct conditioned differential responses are possible in the presence of only one cortical acoustic area. Evidently either hemisphere can be used for problems involving associated memory, and Pavlov's work suggests that both hemispheres are essential for the localization of the direction of a sound source.

SUMMARY

Successive electrical stimuli applied to symmetrical local groups of nerve fibers in opposite ears of the anesthetized dog caused an absolutely unresponsive period lasting 20–100 msec. in the cerebral cortex, followed by an interval of 100–250 msec. during which the second response gradually returned to its original size. The absolute and relative unresponsive periods agreed in duration with the periods obtained to successive stimulation of the same point in one cochlea.

The responsive areas in one cerebral hemisphere to contra-lateral and ipsi-lateral stimulation of symmetrical groups of nerve fibers, corresponded in latency, duration, wave form and initial sign. The amplitudes of the contra-lateral responses were slightly greater than those of the ipsi-lateral.

Records from the radiations and the region of the medial geniculate body showed the same durations of unresponsiveness as seen in the cortical records. Below the medial geniculate body, no responses were noted having greater unresponsive periods for successive stimulation of the same point than 1–2 msec.

In the third acoustic area, successive stimulation of non-symmetrical or identical points in the same or opposite ears, showed only addition of potentials.

This evidence suggests the presence of a neurone common to both ears from the medial geniculate body to all three acoustic areas of the cerebral cortex from each symmetrical group of nerve fibers in opposite cochleas.

REFERENCES

- (1) ALLEN, W. F. *This Journal* **144**: 415, 1944.
- (2) COPPEE, G. E. *Compt. rend. Soc. de biol.* **130**: 1364, 1939.
- (3) BISHOP, G. H. AND J. L. O'LEARY. *J. Neurophysiol.* **3**: 308, 1940.
- (4) GALAMBOS, R. AND H. DAVIS. *J. Neurophysiol.* **6**: 39, 1943.
- (5) HARTLEY, R. V. L. AND T. C. FRY. *Phys. Rev.* **18**: 431, 1921.
- (6) VON HORNOSTEL, E. M. *The Physical Society Discussion on Audition. The Univ. Press, Cambridge.* 120, 1931.
- (7) KEMP, E. H., G. E. COPPEE AND E. H. ROBINSON. *This Journal* **120**: 304, 1937.
- (8) KEMP, E. H. AND E. H. ROBINSON. *This Journal* **120**: 316, 1937.
- (9) LANE, C. E. *Phys. Rev.* **26**: 401, 1925.
- (10) MARSHALL, W. H. AND S. A. TALBOT. *Proc. Am. Physiol. Soc.* 121, 1940.
- (11) MARSHALL, W. H., C. N. WOOLSEY AND P. BARD. *J. Neurophysiol.* **4**: 1, 1941.
- (12) MARSHALL, W. H. *J. Neurophysiol.* **4**: 25, 1941.
- (13) PAVLOV, I. P. *Conditioned reflexes.* Oxford Univ. Press, London, 1928.
- (14) RAYLEIGH, SIR. *Phil. Mag.* **13**: 214, 1907.
- (15) STEVENS, S. S. AND E. B. NEWMAN. *Proc. Nat. Acad. Sci.* **20**: 593, 1934; *Am. J. Psychol.* **48**: 297, 1936.
- (16) STEWART, G. W. *Phys. Rev.* **15**: 432, 425, 1920; **9**: 502, 509, 1917.
- (17) TALBOT, S. A. AND W. H. MARSHALL. *Proc. Am. Physiol. Soc.* 279, 1941.
- (18) TUNTURI, A. R. *This Journal* **141**: 397, 1944; **144**: 389, 1945.
- (19) WOOLSEY, C. N. AND E. M. WALZL. *Bull. Johns Hopkins Hosp.* **71**: 315, 1942.

THE RELATION OF TEMPERATURE AND THE THYROID TO MAMMALIAN REPRODUCTIVE PHYSIOLOGY¹

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Both light and temperature have long been recognized as effective factors in reproduction. It has been shown that the gonads of birds respond to the amount and intensity of light. Rowan (1925, 1938) proved that light, rather than temperature, affects seasonal reproductive physiology in the junco because these birds showed marked sexual development in December at temperatures as low as -52°F . if ample light was supplied. Bissonnette (1930, 1931) using starlings, has confirmed the work of Rowan. According to Benoit (1935), light stimulates the anterior pituitary through the eyes and optic nerves. Bissonnette (1932) reported that ferrets also show reproductive responses to light while Baker and Ranson (1932) report similar responses by field mice.

Some animals respond more readily to temperature changes than to variations in the intensity or duration of light. The reproductive cycle and spermatogenesis in a fish, the three spined stickle-back, was shown by Craig-Bennet (1931) to be conditioned by changes in temperature. Seasonal sexual changes in the male of the thirteen-lined ground squirrel were attributed to some factor other than light by Wells (1935), since the sex rhythm in the male was the same in the dark burrow as in animals exposed to light in the laboratory. Wells (1935) states that during the season of low reproductive activity the testes of the ground squirrel are functional and that they respond to gonadotropins which stimulate spermatogenesis and the production of the testis hormone.

In the farm animals the most marked seasonal change in the physiology of the reproductive organs occurs in sheep. During those months of the year when the environmental temperature is the highest, fertility in the male is often impaired to such an extent that some rams become completely sterile. This infertility associated with high summer temperatures ("summer sterility") was first studied by McKenzie and Berliner (1937). They determined the type and extent of the changes occurring in the reproductive organs and their secretions which are associated with seasonal changes. They observed pronounced reduction in spermatogenesis which was reflected in a decreased number of spermatozoa and a striking increase in the percentage of morphologically abnormal spermatozoa in semen from some rams during the periods of high temperature. Also when they kept rams in a room maintained at high temperature during the normal breeding season (fall and winter) changes occurred in reproductive function similar to those observed during the summer.

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It has been shown (Phillips and McKenzie, 1934; Moore, 1924) that the scrotum is a thermo-regulatory organ which enables the testes to remain at a temperature below that of the body. This lower temperature is essential for proper testicular function as demonstrated by a lack of function when the testis temperature is raised by insulation of the scrotum.

It has been considered that high temperatures during the summer might reduce fertility by the direct effect of temperature upon the testis. However, Berliner and Warbritton (1937) obtained data which indicated that the high temperature was acting through the thyroid and that a hypothyroid state in the summer as the result of the high temperature might cause the impairment in reproductive capacity.

In the spring of 1943, thiourea, thiouracil and similar substances were found to be goitrogenic. Through the action of these goitrogenic agents which prevent thyroxine production and that of thyroxine or thyro-active iodinated proteins, one may now increase or decrease the supply of thyroxine to the animal's body at will. Consequently, in June, 1943, this investigation on the relation of temperature and the thyroid to reproductive physiology in the ram was initiated.

MATERIALS AND METHODS. Two thyro-active materials, crystalline thyroxine and iodinated casein, and the goitrogenic substance, thiouracil, were used to control the level of thyroxine in the body of the experimental rams. Semen was collected each week from 17 different rams with an artificial vagina and the laboratory examinations of the semen specimens were made within an hour after collection. Precautions were taken to prevent changes in the semen between the time of collection and the time the laboratory studies were made.

The following semen characteristics were studied: semen volume, spermatozoan concentration, number of spermatozoa per ejaculate, motility, percentage of morphologically abnormal cells. The percentage of live spermatozoa in the semen as collected, that remaining after 10 minutes' exposure to a temperature of 0°C., and the percentage surviving a storage period of 48 hours were also determined. Since the semen volume, number of spermatozoa per ejaculate, and the percentage of morphologically abnormal cells are sufficient for one to see the general physiological trends associated with temperature and treatment, only the data on these characteristics are presented.

RESULTS. 1. *Influence of high summer temperatures on the characteristics of semen from untreated rams and from rams receiving thyro-active materials.* High summer temperatures cause a lowering in the number of spermatozoa per ejaculate (table 1) and in semen volume (table 2) and an increase in percentage of abnormal spermatozoa (table 3). The progressive changes in each of these semen characteristics indicate a gradual impairment in the activity of the reproductive organs of these rams. When thyroxine was injected or thyro-active iodinated casein was fed, the reproductive capacities of the rams improved as shown by the results of the evaluation techniques. Thus, the semen volume steadily decreased (table 2) and the percentage of abnormal spermatozoa markedly increased (table 3) in the semen from the control rams during August and early September, whereas the semen from those rams receiving thyro-active materials

either showed an increase in semen volume (table 2) and in percentage of normal spermatozoa (table 3) or no further decrease in these semen characteristics was observed after the initiation of thyroxine administration.

Thus thyroid-active substances were therapeutically effective in removing most of the effects of high environmental temperature upon the reproductive

TABLE 1

Influence of high summer temperatures on total spermatozoa per ejaculate in untreated and in rams treated with thyroid-active materials

PERIOD OF JULY 5 TO AUGUST 2. NO TREATMENT IN EITHER GROUP			PERIOD OF AUGUST 2 TO SEPTEMBER 13. GROUP I RECEIVED THYROID-ACTIVE MATERIALS*. GROUP II NO TREATMENT		
Date	Group I, 8 rams	Group II, 4 rams	Date	Group I, 8 rams	Group II, 4 rams
July 5	822,190†	1,678,810	Aug. 9	1,536,175	1,630,625
July 12	551,837	1,071,435	Aug. 16	2,059,938	2,253,750
July 19	363,917	205,100	Aug. 23	1,892,114	780,125
July 26	494,503	448,200	Aug. 30	1,231,438	
Aug. 2	444,830	669,760	Sept. 6	1,308,438	335,400
			Sept. 13	1,501,563	

* Three rams each received 1 mgm. crystalline thyroxine daily by subcutaneous injection. Five rams each received 1 gram iodinated casein per day.

† Expressed in thousands of spermatozoa per ejaculate.

TABLE 2

*The influence of high summer temperatures upon the volume of semen per ejaculate from untreated rams and rams receiving thyro-active materials**

PERIOD OF JULY 5 TO AUGUST 2. NO TREATMENT IN EITHER GROUP			PERIOD OF AUGUST 2 TO SEPTEMBER 13. GROUP I RECEIVED THYROID-ACTIVE MATERIALS. GROUP II NO TREATMENT		
Date	Group I, 8 rams	Group II, 4 rams	Date	Group I, 8 rams	Group II, no treatment
	cc.	cc.		cc.	cc.
July 5	1.34	2.03	Aug. 9	0.73	0.75
July 12	0.85	1.29	Aug. 16	0.70	0.75
July 19	0.81	0.56	Aug. 23	0.70	0.55
July 26	0.75	0.83	Aug. 30	0.57	0.40
Aug. 2	0.60	0.92	Sept. 6	0.70	0.20
			Sept. 13	0.50	0.23

* Three rams each received 1 mgm. crystalline thyroxine daily by subcutaneous injection. Five rams each received 1 gram iodinated casein per day.

organs of these rams. One might conclude then that the temperature is influencing the testes indirectly rather than directly, and that the indirect effect of high temperature is through the thyroid gland.

The histological evidence strikingly substantiates the data presented in the tables. The testis from a normal ram during the fall breeding season shows marked spermatogenic activity and the lumen of the seminiferous tubules is usually filled with spermatozoa (fig. 1). However, after prolonged periods of

high summer temperatures, some rams, particularly those showing marked fattening tendencies, are so affected by the high temperature that derangement

TABLE 3

Influence of high summer temperatures on the percentage of morphologically abnormal spermatozoa in the semen of untreated and thyroxine-injected rams

PERIOD OF JULY 5 TO AUGUST 2. NO TREATMENT IN EITHER GROUP			PERIOD OF AUGUST 2 TO SEPTEMBER 13. GROUP I. 1 MCG. THYROXINE DAILY. GROUP II. NO TREATMENT		
Date	Group I, 3 rams percentage of abnormal sperm*	Group II (4 rams), percentage of abnormal sperm*	Date	Group I, (thyroxine) percentage of abnormal sperm*	Group II (controls), percentage of abnormal sperm*
July 5	37	39	Aug. 9	38	67
July 12	31	32	Aug. 16	38	68
July 19	31	34	Aug. 23	28	71
July 26	48	69	Aug. 30	31	76
Aug. 2	40	48	Sept. 6	30	70
			Sept. 13	29	90

* Each percentage figure represents the average obtained upon two successive ejaculations from all the rams of the group for the particular date designated.

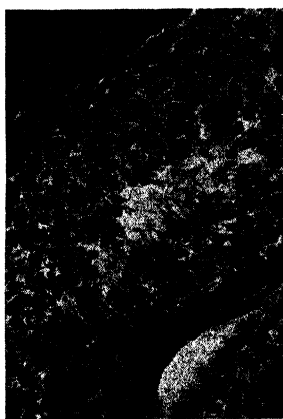


Fig. 1



Fig. 2



Fig. 3

Fig. 1. Section of the ram testis removed during the normal breeding season two weeks preceding the initiation of the oral thiouracil administration. High magnification to show active spermatogenesis and presence of spermatozoa in the lumen of the seminiferous tubule.

Fig. 2. Testis of a Shropshire ram with "summer sterility" during the period of high summer temperature. Note the absence of spermatogenic activity and of spermatozoa.

Fig. 3. Abnormal spermatozoa in a semen specimen from a ram during high summer temperatures. The tailless and coiled tail types are particularly noticeable.

occurs in the spermatogenic tissue and a complete absence of spermatozoa in the lumen of the seminiferous tubules exists (fig. 2).

One infertile ram was administered thyro-active iodinated casein orally at a dosage level of one gram per day. This material contained approximately 2.0

per cent d-l thyroxine. The motility of the spermatozoa, percentage of live spermatozoa (Lasley, Easley and McKenzie, 1942), and the percentage of morphologically abnormal spermatozoa were determined for a pre-treatment period and during the period of administration of the thyro-active material. A marked increase in the motility and percentage of live cells occurred in a short time and normal values for these characteristics were obtained with a treatment period of one month (table 4). The percentage of morphologically abnormal spermatozoa also decreased markedly (table 4 and figs. 3 and 4) during this period.

This ram was mated to each of 5 ewes toward the end of the treatment period. Three of the 5 ewes became pregnant and delivered a total of 5 lambs.

TABLE 4

Influence of orally administered thyro-active protein upon "summer sterility" or the detrimental effects of high summer temperatures

SEMEN CHARACTERISTICS	PRE-TREATMENT PERIOD, AUGUST 13-21	ORAL ADMINISTRATION OF 1 GM. THYRO-ACTIVE PROTEIN AUGUST 21 TO SEPT. 21			
		Aug. 27	Aug. 29	Sept. 4	Sept. 25
Motility	1+	3	4	5	5+
Percentage live spermatozoa	10		71	80	85
Percentage abnormal spermatozoa	33	30	24	15	15

TABLE 5

Changes in semen characteristics associated with the daily injection of 2.5 grams thiouracil
Note similarity of effects produced by thiouracil with those resulting from high summer temperatures

SEMEN CHARACTERISTICS	PRE-TREATMENT PERIOD	PERIOD OF THIOURACIL ADMINISTRATION
Volume (ml.)	0.3	0-0.1
Spermatozoa per ejaculate	618,000,000	16,000,000
Motility	3	0
Percentage abnormal spermatozoa	39	90

II. *Influence of thiouracil administration during the normal breeding season.* Thiouracil was given during the fall breeding season to 3 normal rams. One ram received daily injections of 2.5 mgm. thiouracil, the second ram received by oral administration 10 grams of thiouracil per day, while the third ram was given the same thiouracil treatment (2.5 grams per day) as the first ram and, in addition, was given varying quantities of thyro-active protein orally. The thyro-active protein was given to determine whether the effects of the thiouracil on reproduction were due to its toxic effects or to the hypothyroid state induced by it.

The daily injection of 2.5 gm. thiouracil caused a reduction in the semen volume, spermatozoan motility and the number of spermatozoa per ejaculate and an increase in the percentage of abnormal spermatozoa (table 5). The effect

of the thiouracil, administered during the normal breeding season, was to induce a reproductive condition similar to that observed during periods of high summer temperature. The comparative testicular changes are shown in figure 5 and figures 1 and 2. The testes of the ram injected with thiouracil (fig. 5) and those of the rams affected by high summer temperature (fig. 2) show a similar histological condition, viz. a derangement in the germinal epithelium and a lack of spermatozoa.

Oral administration of thiouracil at the level of 10 gm. per day had less effect upon reproduction of the ram than the daily injection of 2.5 gm. A reduction

TABLE 6

Changes in characteristics of semen associated with daily oral administration of 10 grams thiouracil

These changes are similar to those during periods of high summer temperatures but are less marked than those associated with the daily injection of 2.5 grams thiouracil.

SEMEN CHARACTERISTICS	PRE-TREATMENT PERIOD	PERIOD OF ORAL ADMINISTRATION OF 10 GRAMS THIOURACIL DAILY
Volume (ml.).....	0.35	0.30
Spermatozoa per ejaculate.....	1,121,000,000	600,000,000
Motility.....	3	1
Percentage abnormal spermatozoa.....	24	65

TABLE 7

The influence on the semen characteristics of the simultaneous administration of thiouracil (daily injection of 2.5 grams) and thyro-active protein during the normal breeding season

SEMEN CHARACTERISTICS	PRE-TREATMENT PERIOD	THIOURACIL, 2.5 GRAMS INJECTED DAILY PLUS THYRO-ACTIVE PROTEIN ADMINISTERED IN DAILY DOSAGE OF:	
		2 grams daily	4 grams daily
Volume (ml.).....	0.6	0.3	0.5
Spermatozoa per ejaculate.....	1,347,000,000	578,000,000	1,397,000,000
Motility.....	2	0	1+
Percentage abnormal spermatozoa.....	36	86	64

in the semen volume, number of spermatozoa per ejaculate, and in spermatozoan motility accompanied by an increase in the percentage of abnormal spermatozoa followed the feeding of 10 gm. of thiouracil per day (table 6). The effects on testicular structure are shown in figure 6. The degenerative changes in the seminiferous tubules occurring in the testes of the ram receiving the daily injection of 2.5 gm. thiouracil are less noticeable but there is apparently a cessation of spermatogenesis (fig. 6).

When thyro-active protein was fed simultaneously with the daily injections of 2.5 gm. thiouracil, the effects of the thiouracil on reproduction were lessened. There was a reduction in volume, spermatozoa per ejaculate, and motility and an increase in percentage of abnormal spermatozoa when daily dosage of thyro-

active protein was only 2.0 gm. and that of thiouracil was 2.5 gm. (table 7). When the thyro-active protein was increased to 4 gm. per day, there was an increase in volume, spermatozoa per ejaculate, and motility and a decrease in percentage of abnormal spermatozoa (table 7) but figures equal to the pre-treatment period were not obtained.

SUMMARY AND DISCUSSION. The method whereby seasonal changes influence reproductive physiology in rams differs from that causing similar changes in avian reproductive physiology. In birds, light stimulates reproductive processes indirectly through the pituitary; therefore, birds show an increase in reproductive

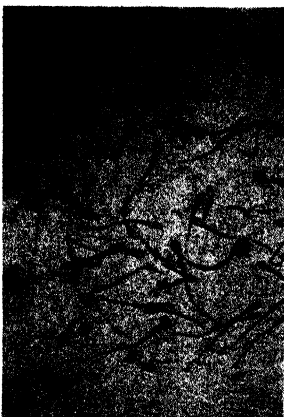


Fig. 4



Fig. 5

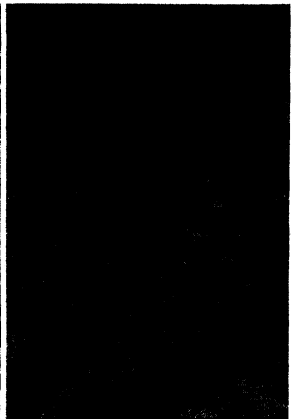


Fig. 6

Fig. 4. Spermatozoa in a semen specimen from the same ram (fig. 3) after the oral administration of thyro-active protein for a two-week period. Notice the decrease in abnormal cells.

Fig. 5. Testis of ram receiving daily injection of 2.5 grams thiouracil. Active degenerative changes are apparent in the seminiferous tubules. Compare with figures 1 and 2.

Fig. 6. Section of right testis removed after the oral administration of thiouracil for 30 days. Cessation of spermatogenesis is apparent, but the degenerative changes in the seminiferous tubules, which occurred after thiouracil injection (fig. 5), are absent. The comparison of this with figure 1 demonstrates thiouracil effects.

activity associated with increasing intensity and duration of light (Rowan, 1925, 1938; Bissonnette, 1930, 1931; Benoit, 1935). In contrast, the ram exhibits the lowest activity of the reproductive organs during periods of greatest intensity and duration of light.

Activity of the organs of reproduction in rams seems to be influenced more by temperature than by light, but the temperature-reproduction interrelation in rams is apparently unlike that in the fish (Craig-Bennett, 1931) and ground squirrel (Wells, 1935; Moore et al., 1934). In the fish and ground squirrel reproductive activity becomes greater with increasing temperatures following the winter period whereas reproductive activity in the ram increases with a lowering of environmental temperature (McKenzie and Berliner, 1937).

A definite lowering in reproductive organ activity of the ram is associated with high environmental temperatures as shown by the results of McKenzie and

Berliner (1937), the present investigations, and other studies. That this temperature effect is not entirely associated with light has been proved by McKenzie and Berliner (1937), who observed a lowering in fertility of rams during the normal breeding season when the rams were subjected to high environmental temperature by keeping them in a heated room.

Although the work of Moore (1924) and Phillips and McKenzie (1934) demonstrated that raising the temperature of the testis to or near body temperature caused a reduction in testicular function, much of the lowered fertility in rams apparently is not caused by the temperature acting directly upon the testis. Temperatures during the summer which will cause a lowering in reproductive capacity in rams may be below that at which the testes normally function during the breeding season. Also, the administration of thyroxine or thyro-active protein during summer causes an improvement in reproductive capacity even during periods of high environmental temperature.

The restoration of a high reproductive level during the summer by administration of thyro-active substances indicates that the rate of thyroid hormone production varies with temperature. In addition, a reproductive condition which is similar to that associated with high summer temperature may be created by reducing or preventing thyroid production through thyroidectomy (Berliner and Warbritton, 1937) or by feeding thiouracil. This latter observation demonstrates that the level of thyroid hormone production influences reproductive processes. When little or no thyroid hormone is produced, reproductive capacity is reduced to a low level and complete sterility may obtain.

It appears, then, that seasonal changes affect reproductive activity through temperature changes which control the rate of thyroid hormone production. A reduction in the rate of thyroid hormone production is apparently associated with high temperatures, and causes a lowering in the activity of the reproductive organs. We, therefore, have an indirect seasonal influence on reproduction in the ram since the seasonal temperature changes affect the thyroid gland and the variations in reproductive physiology are the result of altered thyroid function.

CONCLUSIONS

High temperatures cause a marked lowering in the activity of the reproductive organs of rams.

Thyroxine or thyro-active proteins given to rams during periods of high temperature stimulate the reproductive organs and restore most of the reproductive activities to a level near that of the breeding season.

Changes in semen characteristics similar to those resulting from high environmental temperatures are induced during the breeding season by administration of thiouracil. Thyro-active materials counteract the detrimental effects of thiouracil upon reproduction.

Since the temperatures which will reduce fertility in the ram are below the temperature at which the testes normally function, and since thyro-active materials restore reproductive activity during periods of high temperature, it

is concluded that temperature is not influencing reproductive physiology by its direct effect upon the testes.

The stimulating effect of thyroxine during periods of high temperatures and the harmful effect of thiouracil administered during the normal breeding season suggest that the level of thyroid function influences the relative activity of the reproductive organs.

REFERENCES

- BAKER, J. R. AND R. M. RANSON. *Proc. Roy. Soc. London* **110**(B): 313, 1932.
BENOIT, J. *Compt. Rend. Acad. Sci.* **201**: 359, 1935.
BERLINER, V. AND V. WARBRITTON. *Proc. Am. Soc. Anim. Prod.* 137, 1937.
BISSONETTE, T. H. *Am. J. Anat.* **45**: 289, 307, 1930. *J. Exper. Zool.* **58**: 281, 1931. *Proc. Roy. Soc. London* **110**(B): 322, 1932.
CRAIG-BENNET, A. *Phil. Trans. Roy. Soc. London, ser. B.* 1931.
LASLEY, J. F., G. EASLEY AND F. F. MCKENZIE. *Mo. Agric. Exp. Sta. Cir.* 292, 1942.
MCKENZIE, F. F. AND V. BERLINER. *Mo. Agric. Exper. Sta. Res. Bul.* 265, 1937.
MOORE, C. R. AND W. J. QUICK. *This Journal* **68**: 71, 1924.
PHILLIPS, R. W. AND F. F. MCKENZIE. *Mo. Agric. Exp. Sta. Res. Bul.* 217, 1934.
ROWAN, W. *Nature* **115**: 494, 1925. *Biol. Reviews* **13**: 374, 1938.
WELLS, L. J. *Anat. Record* **62**: 409, 1935.

HEMODYNAMICS IN PULMONARY IRRITANT POISONING

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Pulmonary irritation and pathology incident to phosgene poisoning may produce marked circulatory disturbance not unlike that seen in shock. Many of the circulatory effects after gassing with phosgene were described in papers by Underhill (24), Eyster and Meek (7), Barcroft (1), Laqueur and Magnus (16), and others shortly after World War I. Work during World War II has largely confirmed and amplified these findings. In the recent studies, primary consideration was given to an elucidation of the rôle of the circulation in phosgene death and to the development and evaluation of therapeutic procedures based on the circulatory findings. It is the purpose of the present paper to describe a series of recent investigations on hemodynamics in phosgene poisoning. Possible mechanisms contributing to the circulatory disturbance and the therapeutic implications of the circulatory changes will be considered in some detail. Considerable work here and elsewhere was done with diphosgene as well as with phosgene. Since, so far as we know, the physiologic and pathologic effects of these gases are identical, for convenience reference will be made only to phosgene.

METHODS. A number of circulatory measurements were made on both anesthetized (barbital, nembutal) and unanesthetized dogs before and after exposure to phosgene in concentrations of the order of 0.4 to 0.7 mgm. per liter for 30 minutes. The indices studied included pulse, arterial and venous blood pressures, right ventricular pressure, circulation time and arterial-venous oxygen difference. Hematocrit was also followed. In most dogs, measurements were made at rather frequent intervals, every two to four hours, until death or recovery. All the determinations were not necessarily made on the same dogs.

Arterial and venous blood pressures were usually obtained by needling the femoral artery and vein. In some of the early experiments, pressures were measured in the exteriorized carotid artery and external jugular vein. Pressure was recorded with a mercury manometer for the artery and a water manometer for the vein. Right ventricular pressure was measured by inserting a 3 inch, 18 gauge hypodermic needle through the intact chest wall into the right ventricle (6). The needle was attached to a pressure bottle and mercury manometer. This simple maneuver permitted repeated ventricular pressure measurements even on the unanesthetized animal. In some dogs, especially after frequent pressure measurements, some hemopericardium was evident at autopsy.

Pulmonary circulation time was determined by time measurement from antecubital vein to carotid body after cyanide injection (23), or from vein to tongue

¹ This work was done as part of a contract, recommended by the Committee on Medical Research, between the office of Scientific Research and Development and the University of Chicago.

after fluoresceine (15). With cyanide the first gasp was taken as the end point, with fluoresceine the first appearance of tongue fluorescence was used. The cyanide method proved more convenient and was used exclusively after preliminary experiments. Circulation time in the systemic circuit was indicated by the arterial-venous oxygen difference. Normal saturation differences center around 25-30 per cent and are influenced by degree of tissue activity and rate of blood flow. In the absence of increased tissue metabolism, an increase in arterial-venous oxygen difference must be attributed to a slower blood flow. Arterial and venous oxygens were determined with the Millikan oximeter (19). A simple technique was devised to facilitate its use on dogs. This involved the anaerobic sampling of arterial and venous blood into a specially constructed, thin-walled glass chamber which could be inserted into the oximeter ear-piece (8). The oximeter-chamber technique was calibrated with the Van Slyke-Neill manometric gas analysis apparatus (21).

Circulatory changes were followed not only in untreated, gassed dogs (controls) but also in dogs subjected to various experimental and "therapeutic" procedures. These included unilateral gas exposure, that is, only one lung gassed, the other being protected by a removable plug in the bronchus, with the gassed lung plugged shortly thereafter to keep fluid from the protected side; bleeding—1 per cent of body weight; infusion of saline or plasma - 1 per cent of body weight; bleeding and infusion; and oxygen inhalation - 50 to 100 per cent.

In a few dogs, scleral vessels were directly visualized as a means of exploring the possibility of vascular spasm or red cell agglomeration after phosgene poisoning.² This technique was also applied to gassed rats. Autopsies were performed on most of the fatalities and on sacrificed survivors. Findings relevant to the circulation are reported here.

RESULTS. Pulse. Heart rate fell precipitously with gassing, then slowly rose to or above the initial value (fig. 1). The rather high pre-exposure pulse seen in figure 1 may be due to excitement, since these readings were made on unanesthetized dogs. The early bradycardia is probably a reflex response to irritation of the respiratory passages. It was not observed following bilateral vagotomy or atropinization. No clear difference in pre- or post-gassing pulse was seen between the various groups, based on survival time or treatment (tables 1A and 1B). Comparable tachycardias were encountered in surviving and dying animals. This is shown in figure 2. Although the tachycardia observed in the later stages after gassing is probably partly due to the severe anoxia, no clear relationship was found between the degree of anoxia and extent of the tachycardia. Heart rate sometimes became irregular and slower as death approached, but respiration failed before circulation, the heart continuing to beat for some minutes after respirations ceased.

Arterial, venous and right ventricular pressures. Arterial pressure fell distinctly and progressively with time after gassing. The fall in arterial pressure was sharper in the short survival groups. Venous pressure presented no signifi-

² Kindly done by Dr. Melvin Knisely, Department of Anatomy, University of Chicago, and Dr. M. E. Bloch, Michael Reese Hospital, Chicago.

cant or consistent change. In some dogs venous pressure rose slightly above the normal, in others pressure fell somewhat with time. Composite arterial and venous blood pressure curves are presented in figures 1 and 2. In unanesthetized or barbitalized dogs arterial pressure sometimes dropped to half normal immediately after exposure to phosgene. In morphinized dogs, however, the immediate fall in pressure was slight (table 2). In the later stages after gassing arterial pressure usually decreased to 70–80 mm. Hg. In survivors pressure then recovered slowly, in fatal cases it sometimes continued to fall to as low as 35 mm. Hg shortly before death. The early fall in arterial pressure may be a consequence of the concomitant bradycardia or may be due, in part, to

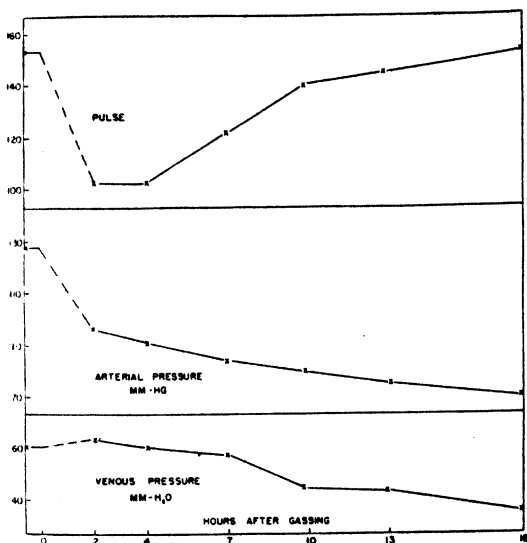


Fig. 1

Fig. 1. Pulse, arterial and venous pressure after phosgene—6 dogs.

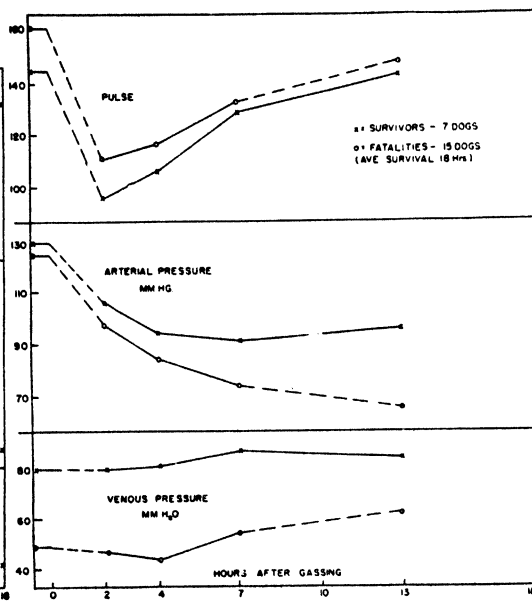


Fig. 2

Fig. 2. Comparison of pulse, arterial and venous pressure in survivors and fatalities.

a reflex peripheral vasodilatation resulting from lung irritation or congestion in the pulmonary circuit. Blood volume was decreased some 50 per cent at 10 hours after gassing (8). This, along with anoxic paralysis of the vasomotor centers, is probably primarily responsible for the late arterial hypotension.

Although we found no marked or consistent increase in venous pressure, the presence of passive congestion, resulting perhaps from derangement of the pulmonary circulation, was suggested in animals dying of phosgene poisoning. Such findings as congestion of the abdominal viscera, distention of the splanchnic vessels, dark red color of the liver, engorged spleen and dilated right heart, were occasional but not consistent findings at autopsy. In a series of 26 dogs, there was no evidence of a rise in right ventricular pressure following gassing, even in one-lung gassed and plugged animals where one would expect considerable

TABLE 1A
Summary data

GROUP	HOURS LIVED	NO. OF DOGS	AT DEATH						2 HRS. BEFORE DEATH					
			Ht.	Art. O ₂ , %	Ven. O ₂ , %	Art. press.	Ven. press.	Pulse	Ht.	Art. O ₂ , %	Ven. O ₂ , %	Art. press.	Ven. press.	Pulse
				Sat'n.	sat'n.		Sat'n.			sat'n.				
						mm. Hg.	mm. H ₂ O					mm. Hg.	mm. H ₂ O	
Control.....	25*	6(3)†	61	43	11	71	79	141	60	52	18	72	61	137
Bled.....	16	7(6)	59	64	3	28	73	154	55	68	12	54	54	148
Infused.....	28	2(2)	72	40	7			86	72	48	11			102
Bled and infused...	24	7(4)	53	35	6			146	49	45	12	97		132
			4 HRS. BEFORE DEATH						6 HRS. BEFORE DEATH					
Control.....	25*	6(3)†	58	60	26	73	50	132	57	67	28	74	32	129
Bled.....	16	7(6)	53	73	24	75	38	140	50	80	31	91	34	139
Infused.....	28	2(2)	73	49	(8)	(38)	(75)	116	73	50	(7)	43	(70)	129
Bled and infused....	24	7(4)	46	56	16	81	79	125	48	65	20	88	103	128

* Survivors counted as 30 hours.
† Died, used for "before death" averages.

TABLE 1B
Summary data

GROUP	HOURS LIVED	NO. OF DOGS	INITIAL						2 HRS. AFTER GAS					
			Ht.	Art. O ₂ , %	Ven. O ₂ , %	Art. press.	Ven. press.	Pulse	Ht.	Art. O ₂ , %	Ven. O ₂ , %	Art. press.	Ven. press.	Pulse
				sat'n.	sat'n.		sat'n.			sat'n.				
				<i>mm.</i> <i>Hg</i>	<i>mm.</i> <i>H₂O</i>		<i>mm.</i> <i>Hg</i>			<i>mm.</i> <i>H₂O</i>				
Control.....	25*	6 (3)†	44	88	60	127	61	153	45	80	45	96	64	102
Bled.....	16	7 (6)	43	86	59	134	61	153	45	80	48	110	42	115
Infused.....	28	2 (2)	57	87	55	114	45	168	57	81	43	92	48	116
Bled and infused...	24	7 (4)	41	87	61	128	67	154	42	81	41	98	67	97
			4 HRS. AFTER GAS						7 HRS. AFTER GAS					
Control.....	25*	6 (3)†	48	80	42	91	61	103	53	80	37	84	58	122
Bled.....	16	7 (6)	48	79	42	85	33	124	53	76	23	63	43	144
Infused.....	28	2 (2)	60	81	40	85	51	126	63	80	36	77	56	134
Bled and infused....	24	7 (4)	44	81	37	90	71	109	48	80	35	93	95	128
			13 HRS. AFTER GAS											
Control.....	25*	6 (3)†	59	76	28	75	44	144						
Bled.....	16	7 (6)	57	69	19	76	57	129						
Infused.....	28	2 (2)	66	66	30	60	65	143						
Bled and infused....	24	7 (4)	51	75	35	90	103	135						

* Survivors counted as 30 hours.
† Died, used for "before death" averages.

vascular obstruction in the plugged lung (table 2, fig. 3). In morphinized animals, right ventricular pressure remained essentially constant; in unanesthetized or barbitalized animals ventricular pressure fell with time, closely parallel to systemic arterial pressure. Venous pressure, nevertheless, sometimes rose. This occasional rise in pressure may be attributed perhaps to a decreased

TABLE 2
Right ventricular, arterial and venous pressures

	PRE-EXPOSURE			% INITIAL AFTER GASSING							
	Rt. vent. press.	Art. press.	Ven. press.	2 hrs.		6 hrs.		12 hrs.		18 hrs.	
	mm./Hg	mm./Hg	mm./H ₂ O								
No drugs or barbital	37 (11)*	109 (8)	55 (6)	87	89	62	57				
					114		114				
Morphine	31 (10)	100 (10)	57 (3)	87	92	80	86	77	76	(80)	(84)
					140		155		145		195
Unilateral gassed and plugged	42 (6)	126 (6)		62	62	71	75	88	91		

* Number of readings averaged.

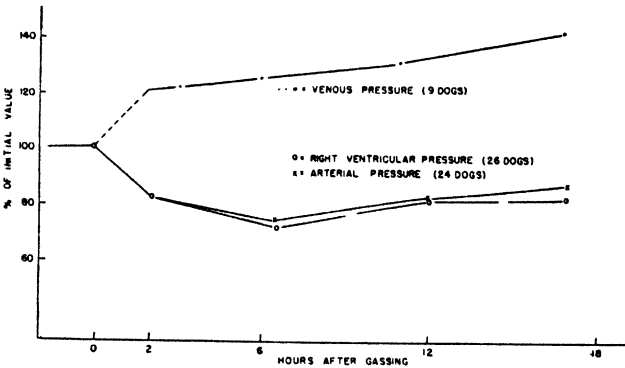


Fig. 3

Fig. 3. Absence of back-pressure in the pulmonary circuit.
Fig. 4. Relation of circulation time to hemoconcentration.

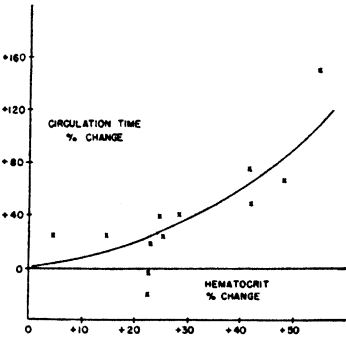


Fig. 4

chest pumping action because of the rapid and shallow breathing or to compression of the intrathoracic veins by the heavy and voluminous lungs, rather than to a cardiac effect.

Circulation time. Pulmonary circulation time was increased on the average by two-thirds at 8 to 12 hours after gassing (table 3). A similar slowing in the systemic circuit was indicated in some dogs by a rising arterial-venous oxygen

difference. Although the latter was almost always low terminally because of the low arterial oxygen, it was often increased 50 per cent or more at 10–12 hours after gassing, from a normal average of 30 per cent saturation difference to a maximum of 60 per cent. The relationship between pulmonary circulation time and hematocrit is shown in figure 4. Circulation velocity was found to vary inversely with hematocrit. An early temporary slowing of the circulation was suggested by the lowered blood pressure and pulse during the first hour or two after gassing.

In vivo vascular visualization. Spasms were seen in the systemic vessels of gassed rats. Vessels, as large as 300 micra diameter, in extensive patches of surviving mesentery, could be seen repeatedly to squeeze themselves empty of blood at intervals of a few seconds to half a minute. Occasional spasms were seen in the scleral vessels of gassed dogs. In vivo clumping or agglomeration of blood was also noted. This was especially severe after heavy exposure to phosgene. Visualization of the superficial pulmonary vessels in dogs indicated a

TABLE 3
Circulation time
(Antecubital vein to carotid sinus—cyanide)

NORMAL	% INCREASE, HOURS AFTER GASSING					
	1	4	6	8	12	24
9.5 sec.*....	10 (3)†	45 (6)	55 (3)	60 (5)	65 (5)	0 (3)

* Range 6.1 to 14.2 in ten dogs; but repeat determinations on six animals (at 24 hr. intervals) agreed within 20 per cent maximum, 12 per cent average.

† Figures in parentheses refer to number of readings averaged.

considerable increase in lung blood soon after gassing, with a subsequent falling off toward normal.

Circulatory failure. The basically asphyxial nature of phosgene death is indicated in figure 5. Gassed animals died when the venous oxygen approached zero, with the arterial oxygen falling roughly in parallel. Death occurred when the hematocrit was rising, maximal or falling, or was at a high or low level. This was true not only for untreated, gassed animals but also for those bled, infused with saline or plasma or breathing oxygen. This is not to say, however, that a failing circulation was of no significance in leading to the final breakdown. Circulatory disturbance as indicated by the arterial-venous oxygen difference appeared to have been an important factor in the outcome of 12 of 48 fatalities of all types (untreated, bleeding, infusion, bleeding and infusion, oxygen or unilateral exposure).

In these, the arterial-venous oxygen difference was maximal terminally and reached values of 60 per cent or higher. No untreated animal fell into this group. Circulatory disturbance was frequent in dogs bled subsequent to gassing (60 per cent), exposed unilaterally (50 per cent), or treated with oxygen (35 per cent), table 4. Bleeding, by exaggerating the fall in blood volume, would di-

rectly favor shock. Unilaterally exposed animals survived relatively long and with a functional lung so that death, if it did occur, was likely to result from developing circulatory collapse. Oxygen-treated animals lived on the average twice as long as controls and had for a longer time a higher arterial-venous oxygen difference which, however, fell toward death. The higher incidence of cir-

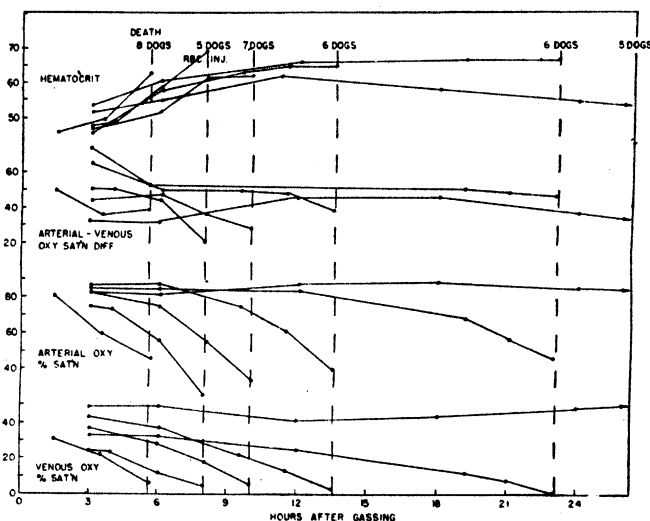


Fig. 5. Relation of hemoconcentration and anoxia to time of death.

TABLE 4

*Incidence of circulatory disturbance as indicated by arterial-venous oxygen difference**

GROUP	NUMBER OF DOGS	IMPAIRMENT OF CIRCULATION		
		Negligible	Moderate	Severe
Untreated	14	12	2	0
Bled.....	7	2	1	4
Bled and transfused or infused.....	9	7	0	2
Infused.....	4	4	0	0
Unilaterally gassed	6	3	0	3
Oxygen treatment.....	8	4	1	3
Total.....	48	32	4	12

* This does not include cases of circulatory impairment associated with severe anoxia, seen in low arterial saturation.

culatory impairment in the oxygen group may not be due entirely to the prolongation of life. Since the criterion for circulatory disturbance was a large arterial-venous oxygen difference, animals with a low arterial saturation were excluded. Oxygen inhalation may unmask some of these with poor circulation.

DISCUSSION. It was thought that one of the ways in which phosgene damage to the lungs might produce systemic effects and death was by interference with

the pulmonary circulation. Early reports described damage of the pulmonary capillary blood vessels after gassing, with intracapillary thrombosis and complete stoppage of the circulation in some areas (1, 11, 13). Recent workers, however, question, on the basis of microscopic evidence, anatomical damage to alveolar capillaries (5, 8, 17).

Likewise, the evidence for lung thrombosis is equivocal (5, 8, 26). Thrombi apparently are not universally distributed in phosgene gassed lungs and their presence or absence might depend upon locus of observation as well as upon gas concentration during exposure. The red cell agglomeration seen in systemic vessels is probably not restricted to the systemic circuit. Mechanical compression of the pulmonary vascular bed by lung fluid must occur to some extent. The spurting of edema fluid that may be seen when a cut is made into a hepatized lung is indicative of considerable pressure by the extra-vascular fluid. Further, mechanical compression by emphysematous blebs, trapped in lungs under increased pressure as a result of probable bronchial obstruction, may be a factor. We have noted many empty capillaries along with engorged ones in the alveolar walls of gassed lungs. Spasms have been seen in the systemic vessels. Such vessel responses may also occur in the pulmonary circuit and contribute to a deranged pulmonary flow.

The preceding considerations indicate that there is some disturbance of the pulmonary circulation after gassing. Whether this is sufficient to interfere with circulation or respiration is another question. If a considerable fraction of the pulmonary vascular bed were occluded, blood flow in the remaining channels might be so rapid that adequate gas exchange could not be maintained, aside from the pulmonary edema itself. Such an effect was obtained by Binger et al. (2) following the production of pulmonary emboli by the injection of appropriate size particles. After phosgene, however, there is a prolonged pulmonary circulation time and, therefore, inadequate oxygenation because of rapid flow can be excluded as a complication.

Clinical experience with phosgene-gassed patients in World War I pointed to right heart embarrassment as a primary factor in the fatal outcome. The presence of enlargement of the right heart, general passive congestion and venous engorgement in the blue or asphyxial cases and their absence in the grey or shock type cases was reported by the majority of clinicians who observed gassed patients (10, 11, 14, 20). Cardiac dilatation and venous engorgement were seen by us and by other workers (7, 26) in animals dying from phosgene, but these were not consistent findings (8, 17). Knisely, who has applied his visualization technique to rats which we have gassed, has seen evidence of back-pressure on the heart. On the other hand, we have found that the isolated lung shows no increased resistance to blood perfusion after being gassed in vitro and while developing a marked edema (8). Further, venous pressures have rarely shown an appreciable increase in our series of gassed dogs. This was also found to be true by others (7, 22). In a recent case, in which venous pressure was measured in a gassed man, no significant elevation was noted (18). Conclusive evidence of the

absence of any back-pressure on the right heart was obtained in a series of 26 gassed dogs. Direct measurement of right ventricular pressure indicated no rise, rather a fall, after gassing. Absence of back-pressure was also recently demonstrated independently by measurement of pulmonary artery pressures in gassed cats (17).

These findings are not surprising, even if appreciable pulmonary vascular interference does exist, for there is considerable evidence that pulmonary vascular obstruction would have to be present in rather extreme degree to increase the pulmonary arterial pressure and embarrass the right heart. Thus, one-lung gassed dogs can survive indefinitely and with no serious circulatory embarrassment. This was true even when the gassed and subsequently plugged lung became turgid with edema fluid and probably possessed little, if any, active circulation. Brenner (4) found that ligation of one pulmonary artery caused only a slight and transient pressure rise behind it. Other experiments in the literature (9, 25), similarly indicate that two-thirds of the pulmonary arterial channels can be occluded without disturbing the right heart or altering arterial and venous pressures and cardiac output.

The absence of any increased back-pressure on the right heart under any type or degree of lung pathology produced in animals by gassing with phosgene is a reflection of the compensatory mechanisms or vascular reserve inherent in the lesser circulation. Despite the apparent beneficial effects of phlebotomy reported from clinical experience in World War I (20), the absence of back-pressure on the heart and the indifferent or even deleterious therapeutic results obtained with bleeding in carefully controlled animal experiments here and elsewhere (5) argue against venesection as a therapeutic maneuver in phosgene cases. The slight hemodilution produced by venesection is not of any material benefit since it has been demonstrated that hemoconcentration is not the primary factor determining death in gassed animals. In fact, harm may be done by late venesection which further reduces a blood volume already lowered by plasma transudation.

Death due to circulatory collapse per se is an infrequent occurrence in animal experiments. Shock therapy-infusion of whole blood, plasma or saline, desoxycorticosterone, vitamins (including anti-capillary permeability entities), salts, etc., have made little difference in the mortality rate of gassed dogs (8). Since circulatory failure unassociated with anoxic anoxia occurred more frequently in late survivors, in oxygen-treated or in unilaterally-gassed animals (5, 8), shock therapy might be more useful at this time. This possibility, however, was not evaluated. There is no evidence of cardiac failure and, therefore, no rational basis for heart stimulants. Clinical reports (3, 12) of benefit from their use must be taken with reserve. Heparin (17) to prevent thrombus formation and quinine or atabrine used by us to prevent clumping have proven therapeutically useless. Histamine has been suspected as a possible toxic substance liberated from injured lungs which might be responsible for some of the systemic effects. Histaminase, however, did not alter the gassing syndrome, nor did it prevent or delay death.

Further, histamine did not accelerate a fatal outcome (8). The possibility that a toxic substance might be responsible for some of the circulatory effects was rendered unlikely by the findings (8) that: edema fluid reaching a normal lung or injected intravenously did little damage; normal dogs were not injured when their own blood was substituted with blood taken from other animals an hour after exposure to a heavy phosgene dose; in cross-circulation experiments (normal cross-circulated with gassed animal) the ungassed dog survived indefinitely with proper post-operative care.

SUMMARY

Some circulatory impairment is evidenced soon after phosgene poisoning by low arterial pressure, prolonged pulmonary circulation time, increased arterial-venous oxygen difference and hemoconcentration with its decreased blood volume and increased blood viscosity. These may contribute to the final break-down by exaggerating the tissue anoxia already present because of the low arterial oxygen saturation. Death, however, is due primarily to an interference with oxygen uptake through edematous lungs. If the acute stage of pulmonary edema with its attendant anoxic anoxia is survived, circulatory failure may become a more important factor in the ultimate outcome.

There is no increased back-pressure on the right heart under any type or degree of lung pathology produced in dogs by gassing with phosgene. This is indicative of a considerable vascular reserve in the pulmonary circuit and eliminates right heart embarrassment as an important complication of gassing.

REFERENCES

- (1) BARCROFT, J. J. Roy. Army Med. Corps **34**: 155, 1920.
- (2) BINGER, C. A. L., G. R. BROW AND A. BRANCH. J. Clin. Investigation **1**: 55, 1924-1925.
- (3) BLACK, J. E., E. T. GLENNY AND J. W. MCNEE. Brit. Med. J. **2**: 165, 1915.
- (4) BRENNER, O. Arch. Intern. Med. **56**: 211, 457, 724, 976, 1189, 1935.
- (5) BUNTING, H. Personal communication.
- (6) DUNN, J. S. Quart. J. Med. **13**: 46, 1919-1920.
- (7) EYSTER, J. A. E. AND W. J. MEEK. Med. Dept. U. S. Army in World War **14**: 356, 1926.
- (8) GERARD, R. W., J. M. TOBIAS, H. M. PATT, M. N. SWIFT, R. CARLSON AND S. POSTEL. Unpublished observations.
- (9) GIBBON, J. H., JR., M. HOPKINSON AND E. D. CHURCHILL. J. Clin. Investigation **11**: 543, 1932.
- (10) GILCHRIST, H. L. Med. Dept. U. S. Army in World War **14**: 250, 1926.
- (11) GILCHRIST, H. L. AND P. B. MATZ. Med. Bull. Vet. Adm. **10**: 1, 1933.
- (12) HALDANE, J. S. J. Roy. Army. Med. Corps **33**: 494, 1919.
- (13) HEGLER, C., F. WOHLWILL AND H. MAYER. Deutsch. Med. Wehnschr. **54**: 1551, 1928.
- (14) HERRINGHAM, W. P. Lancet **1**: 423, 1920.
- (15) LANGE, I. AND L. J. BOYD. Med. Clin. North America **26**: 943, 1942.
- (16) LAQUEUR, E. AND R. MAGNUS. Ztschr. f. d. ges. Exper. Med. **13**: 31, 1921.
- (17) LOCKWOOD, J. Personal communication.
- (18) LONGCOPE, W., M. WINTROBE AND J. LEUTSCHER. Personal communication.
- (19) MILLIKAN, G. A. Rev. of Scient. Instr. **13**: 434, 1942.
- (20) NORRIS, G. W. Trans., College of Physicians. Third Series, **41**: 120, 1919.

- (21) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry, Methods. The Williams & Wilkins Company, Baltimore, 1932.
- (22) Phosgene Workers Meeting, New York, New York, October, 1942.
- (23) ROBB, G. P. AND S. WEISS. Am. Heart J. **8**: 650, 1933.
- (24) UNDERHILL, F. P. Med. Dept. U. S. Army in World War **14**: 313, 1926.
- (25) WINANS, H. M., J. V. GOODE AND C. T. ASHWORTH. South. Med. J. **35**: 225, 1942.
- (26) WINTERNITZ, M. C. Pathology of war gas poisoning. Yale University Press, 1920.

SODIUM CHLORIDE AS AN ADJUNCT TO A DIET OF WHOLE WHEAT AND WHOLE MILK

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From several laboratories have come reports of the effect of chloride and sodium deficiency or excessive ingestion upon carbohydrate metabolism (1), glucose tolerance (2) and the mineral composition (3) of the body. This paper reports only the effect upon growth and reproduction of different levels of sodium chloride.

For years it has been customary in the Columbia laboratories to add to the diets consisting of dried whole milk and whole wheat, sodium chloride to the amount of two per cent of the weight of the wheat. As the per cent of wheat in the diets varied from approximately 33 to 90, the actual amounts of sodium chloride varied from 0.66 to 1.77 per cent of the mixture.

Throughout this period no differences had been observed in the condition of the animals which could be attributed to the variation in sodium chloride, but a short time ago it was decided to set up an experiment in which the amount of wheat and milk in the diet would be constant and the sodium chloride vary more widely. The following is the report of this experiment, with a brief reference to earlier work not carried so far, but in which the per cent of salt varied more widely.

EXPERIMENTAL. Three diets were used containing 1.32, 2.59 and 5.06 per cent of sodium chloride, and they will be referred to as diets 1, 2 and 3 (the laboratory numbers are 13, 130 and 140), diet 1 being the accepted standard for much of our work (4, 5, 6).

The experiment was set up using matched lots of rats, each lot consisting of males and females, kept together at all times, except when females were pregnant or suckling young, so that breeding might occur as freely as possible. In this series the animals were all killed at one year of age and sent to Doctor Halsey of the College of Pharmacy for examination.

Another comparison of the first two diets had been made earlier, and those animals were kept for the complete life cycle, and examined at death by Doctor Sproul of the College of Physicians and Surgeons. Their record to one year of age is included with the above in the following table.

There was no consistent difference in growth on any of these diets up to three or four months of age, beyond that it appears that the animals on diet 3 containing the higher amount of sodium chloride, grew more slowly. It would require a larger number of animals to substantiate this.

As shown in the accompanying table there seemed to be little difference in the

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age at which the females matured, a larger number of young were born and reared on diets 2 and 3, but the weight of the young at weaning was significantly less on diet 3.

In the earlier experiment with the first two diets the complete breeding record of the females on diet 2 was better in every respect.

As stated earlier, the animals killed at one year of age were examined by Doctor Halsey. He reports less kidney fat than usual on this milk-wheat diet, and with regard to the kidneys, says: "The kidneys of these animals with but one exception appeared abnormal at post mortem when viewed microscopically. The abnormalities ranged from a slight paleness and mottling, and the flabbiness and enlargement shown by some of the animals, to the enormously dilated kidneys of one female, which were several times the normal size of the organ, cavernous, and had undergone pressure atrophy to such an extent that both

TABLE 1
Breeding record to one year of age

	DIET 1		DIET 2		DIET 3	
	Num- ber of cases	Mean	Num- ber of cases	Mean	Num- ber of cases	Mean
Age in days at birth of first young.....	15	106.5	12	112.6	9	108.0
Young born per female.....		27.4		30.3		29.0
Young reared per female.....		18.7		23.1		25.2
Per cent of young reared.....		68.1		76.3		87.0
Average weight in grams of young at 28 days.....	280	46.7 \pm 0.19	277	45.3 \pm 0.23	227	41.9 \pm 0.20
C.V.....		10.1		12.7		10.9

cortex and medulla had practically disappeared. Search was made for kidney stones which often are found in cases of hydronephrosis but none were found."

Of the animals on diet 2 in the earlier experiment, which died at the age of 650 to 1077 days of age and were examined by Doctor Sproul, two females and one male were reported as having kidney lesions of some degree of severity. Unfortunately, due to an accident, all the animals killed at one year of age on diet 1 and diet 2 were lost, so there are no reports to compare with that of Doctor Halsey above; but such a high per cent of kidney lesions is unusual, even in older rats which die a natural death. As to the one animal which showed such an enormous dilatation and severe hydronephrosis, that occurs on all diets occasionally and was reported in the case of one of the animals on diet 1 which died at 139 days of age.

In a series of tests carried out several years ago in which the per cent of sodium chloride varied from 0 to 9.09, no difference was noted in growth or breeding record until the per cent of sodium chloride reached the highest figure above, or seven times the usual amount.

SUMMARY

The presence or absence of sodium chloride in diets consisting of whole wheat and dried whole milk appears to make no difference in growth or apparent well-being of the albino rat until the amount reached the level of about 9 per cent. However there is a possibility of some kidney damage at a level of about 5 per cent.

REFERENCES

- (1) LEWIS, R. C., JR., F. S. MCKEE AND B. B. LONGWELL. J. Nutrition **27**: 11, 1944.
- (2) SAYERS G., M. A. SAYERS, AND J. M. ORTEN. J. Nutrition **26**: 139, 1943.
- (3) THACKER, E. J. J. Nutrition **26**: 431, 1943.
- (4) CAMPBELL, H. L. AND H. C. SHERMAN. J. Nutrition **16**: 603, 1938.
- (5) SHERMAN, H. C., H. L. CAMPBELL AND C. S. LANFORD. Proc. Nat. Acad. Sci. **25**: 16, 1939.
- (6) LANFORD, C. S., H. L. CAMPBELL AND H. C. SHERMAN. J. Biol. Chem. **137**: 623, 1941.

EFFECT OF PENTOTHAL ANESTHESIA ON CANINE CEREBRAL CORTEX¹

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Quastel (1) pioneered in the field of barbiturates and brain metabolism and found that barbiturates reduce the oxygen intake of excised cerebral tissues. Himwich, Sykowski and Fazekas (2) noted that the higher parts of the brain are depressed to a greater degree than the lower portions by pentobarbital. However, these results could not be applied directly to the living organism chiefly because the dosage was greater than that used even in the most profound surgical anesthesia. Yet such an application is important in order to determine whether histotoxic anoxia is part of the mechanism of barbiturate narcosis. The first *in vivo* study of barbiturates was made by Schmidt, Kety and Pennes (3) who, using a direct method for the measurement of cerebral blood flow, observed in deeply narcotized monkeys a lower cerebral metabolic rate than in lightly anesthetized animals. Because this observation is fundamental for the theory of narcosis, it was thought worth while to confirm it and extend it to other species. In the present investigation the effect of pentothal narcosis is examined in the dog.

METHOD. In order to measure cerebral blood flow (C.B.F.) without the changes attendant upon operative interference, an indirect method is necessary. In the present study, we used Kety and Schmidt's (4) procedure which depends upon the inhalation of an indifferent gas, nitrous oxide, and its absorption by the brain. With the value for C.B.F. so obtained and the cerebral arteriovenous oxygen (AVO_2) difference, i.e., volume of oxygen consumed by the brain from each 100 cc. of blood passing through that organ, cerebral metabolic rate (C.M.R.) is calculated.

Seven dogs under pentothal anesthesia were prepared by trephination of the cranium over superior longitudinal sinus and exposure of the femoral artery. While an animal was respiring a mixture of 15 per cent nitrous oxide, 21 per cent oxygen, and 64 per cent nitrogen, samples of blood were collected simultaneously from the superior longitudinal sinus, draining the cerebral cortex, and from the femoral artery. The blood was analyzed for oxygen to obtain the AVO_2 difference and for nitrous oxide in order to measure the cerebral blood flow. Determinations were made in two depths of anesthesia: one in which the anesthesia was the lightest possible that would permit manipulations and another in which nocuous stimuli evoked no apparent response.

¹ This investigation was aided by a grant from the Winthrop Research Fund.

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RESULTS. In table 1 we see the effects of minimal and deep barbiturate anesthesia on cerebral AVO_2 difference, C.B.F., and C.M.R. The dog reacts to profound pentothal depression more frequently with a reduction of the AVO_2 difference than with a retardation of C.B.F., for the AVO_2 difference was diminished in every instance by deep anesthesia, while blood flow was impaired in only 4 of the 7 experiments. The average AVO_2 difference fell from 10.0 volumes per cent to 6.0 volumes per cent, the average blood flow decreased from 63 cc./100 grams of tissue/minute to 50 cc./100 grams of tissue/minute, and the oxygen intake diminished from 5.9 cc. oxygen/100 grams of tissue/minute to 2.6 cc. oxygen/100 grams of tissue/minute. Thus, on the average, AVO_2 difference fell 40 per cent and C.B.F. declined 22 per cent; and since C.M.R. is the product of the two, it was decreased to an even greater extent, 56 per cent.

TABLE 1
Effect of pentothal anesthesia on metabolism of cerebral cortex in dog

DOG NO.	LIGHT ANESTHESIA			DEEP ANESTHESIA		
	AVO_2	C.B.F.	C.M.R.	AVO_2	C.B.F.	C.M.R.
1	10.2	72	7.3	6.5	33	2.1
2	11.7	58	6.8	6.1	58	3.5
3	10.1	61	6.2	7.0	45	3.3
4	11.7	34	4.0	5.9	45	2.7
5	8.0	95	7.6	2.0	110	2.2
6	5.4	78	4.2	2.5	30	0.8
7	12.8	43	5.5	11.7	28	3.3
Average	10.0	63	5.9	6.0	50	2.6

The three designations at the head of the columns of figures, AVO_2 , C.B.F., and C.M.R., signify Cerebral Arteriovenous Oxygen difference (volumes per cent), Cerebral Blood Flow (cc./100 grams of tissue/minute), and Cerebral Metabolic Rate (O_2 cc./100 grams of tissue/minute), respectively.

DISCUSSION. These results yield additional evidence for the viewpoint that barbiturates exert a histotoxic anoxic action in the living organism, in this case the dog. Depression of cerebral cellular oxidations is, therefore, a factor in barbiturate narcosis. The metabolic rate under lightest possible anesthesia, 5.9 cc./100 grams of tissue/minute, may be excessive, perhaps because the method used for blood flow tends towards high values. The fact remains, however, that results obtained by the same method both in monkey and in unanesthetized man are lower (3, 5, 6). This comparatively faster rate in the dog may be explained by the fact that the venous blood collected from the dog represented almost entirely the venous return from the cerebral hemispheres, whereas in the monkey and in unanesthetized man, the venous blood had a more diverse origin and included other parts of the brain as well as the cerebral hemispheres. If this fast metabolism in the dog is not caused by a species difference, it may be ascribed to the cortex uncontaminated by subcortical influences. Even in man

with only partial segregation of the cortical blood, the cerebral hemispheres consume a greater volume of oxygen than the other cerebral parts (6). In the dog, however, the excised caudate nucleus exhibits a higher metabolism even than does cortical grey tissue. Perhaps the *in vitro* observations may be compared with the resting metabolism, while the *in vivo* ones include two quotas: one for maintenance and another for function.

SUMMARY

The effect of light pentothal narcosis was compared with that of deep anesthesia and the average oxygen intake in the brain of 7 dogs was found to fall from 5.9 cc. oxygen/100 grams of tissue/minute to 2.6 cc. oxygen/100 grams of tissue/minute, a decrease of 56 per cent. The cerebral metabolic rate is higher than that obtained from the brain of man and monkey using the same methods, and the difference is imputed to the fact that in the dogs the venous blood came chiefly from the cerebral hemispheres which possess a faster metabolism than the lower parts of the brain.

REFERENCES

- (1) QUASTEL, J. H. *Physiol. Rev.* **19**: 135, 1939.
- (2) HIMWICH, H. E., P. SYKOWSKI AND J. F. FAZEKAS. *This Journal* **132**: 293, 1941.
- (3) SCHMIDT, C. F., S. S. KETY AND H. H. PENNES. *This Journal* **143**: 33, 1945.
- (4) KETY, S. S. AND C. F. SCHMIDT. *This Journal* **143**: 53, 1945.
- (5) ETSTEN, B., G. E. YORK AND H. E. HIMWICH. *Arch. Neurol. and Psychiat.* **56**: 171, 1946.
- (6) HIMWICH, W. A., E. HOMBURGER, R. MARESCA AND H. E. HIMWICH. *Fed. Proc.* **5**: 47, 1946.

LIPOTROPIC ACTION OF LIPOCAIC: A STUDY OF THE EFFECT OF ORAL AND PARENTERAL LIPOCAIC AND ORAL INOSITOL ON THE DIETARY FATTY LIVER OF THE WHITE RAT¹

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Hershey's demonstration of the effect of lecithin in preventing fat deposition in the livers of insulin treated depancreatized dogs in 1931 (1) has been followed by an ever increasing amount of investigation of substances causing or preventing fatty livers. In an excellent review of this work, published in 1944, McHenry and Patterson (2) have clearly differentiated the various types of fatty livers which have been produced, and have called attention to the intimate relation of various members of the B complex of vitamins to fat metabolism. They have considered the known lipotropic factors under the following three divisions: I. Choline and related substances and choline precursors such as methionine. II. Lipocaic. III. Inositol.

Lipocaic is the name suggested by Dragstedt, Prohaska and Harms (3) for the specific substance in pancreas which is effective on oral administration in preventing or relieving the fatty infiltration of the liver in insulin treated depancreatized dogs and in making possible their survival. Although there appears to be substantial agreement now that the activity of lipocaic in depancreatized dogs is not due to choline, methionine, or the general lipotropic action of protein, the view of Dragstedt that lipocaic is an internal secretion of the pancreas is still in debate. Thus, Best and Lucas (4) regard it not as a hormone but as "an unidentified substance in pancreas which affects fat metabolism." McHenry and Patterson (2) are reluctant to accept lipocaic as an internal secretion, partly because the signs and symptoms of lipocaic deficiency do not appear for several weeks after pancreatectomy, whereas evidence of insulin deficiency becomes manifest within a few hours. Both groups of workers prefer to regard lipocaic as a specific dietary factor. It should be pointed out in this connection that the relatively long latent period between pancreatectomy and the development of symptoms of lipocaic deficiency does not necessarily contradict the view that lipocaic is an internal secretion since there is a similar long latent period between removal of the thyroid or the gonads and the appearance of deficiency symptoms.

McHenry and Gavin (5) and Gavin and McHenry (6) have demonstrated that both lipocaic and inositol exert a strong lipotropic action in rats fed a fat-free diet plus thiamine, riboflavin, pyridoxine, pantothenic acid and biotin. These workers also report that extracts of liver, muscle, wheat germ and rice prepared according to methods described for the preparation of crude lipocaic

¹ This work has been aided by grants from Mr. Andrew Wigeland, Eli Lilly Company, Armour and Company and from the Josiah Macy Jr. Foundation.

were effective in preventing the biotin type of fatty livers (7) and that preparations of lipocaic examined in their laboratory contained sufficient inositol to account for their lipotropic potency in rats (2). Forbes (8) has confirmed this lipotropic action of inositol in rats on a fat-free diet containing other members of the B complex and has reported that the effect of choline plus inositol is greater than that of either one alone. Both groups of workers preceded their experimental period by a three week period of a diet designed to deplete the animals of stores of B vitamins and fat.

In previous studies from this laboratory (9) it was found that inositol was entirely ineffective in preventing or curing the fatty livers of insulin treated depancreatized dogs even when used in doses as large as some of the active preparations of lipocaic. Since these pancreatic extracts could not conceivably be pure inositol, it seems clear that the activity of lipocaic in the depancreatized dog is not to be accounted for on the basis of the possible presence of inositol.

We have also found (10) that the lipotropic action of lipocaic, in preventing fat deposition in the livers of rats on a high fat, low protein diet containing all B vitamins in the form of brewer's yeast, is due to some substance other than choline, methionine, or protein. The effect of pancreatic extracts containing lipocaic on various types of dietary fatty livers in rats could, however, be due to a substance in the extract different from that effective in the depancreatized dog. For this reason and also because the basal diet and method of conducting the experiment differed so widely from that employed by Gavin and McHenry and by Forbes, it was desirable to determine if inositol would exert an action similar to that of lipocaic under the conditions of our experiment.

EXPERIMENTAL METHODS. White male rats ranging from 115–150 grams in weight were used. They were kept in individual cages and given food and water ad libitum. The basal diet consisted of 5 per cent vitamin test casein, 40 per cent lard, 2 per cent ruffex, 5 per cent modified Osborne Mendel salt mixture, 5 per cent Mead's brewer's yeast powder, 1 per cent cod liver oil and 42 per cent glucose. Lipocaic was prepared from fresh pancreas glands of beef by the method previously described (10). It was essentially a defatted acid-alcohol extract subsequently adjusted to pH 7.0 with calcium oxide.

The pancreas extract was dissolved in water making an 8 per cent solution. A small amount of flocculent material remained insoluble. The mixture was stirred vigorously and 260 cc. mixed with 1300 grams of the basal diet to make up the 1.6 per cent unfiltered lipocaic diet. The remainder was filtered through paper and then passed through a Berkfeld filter, and 260 cc. of this filtrate were mixed with a second 1300 grams of basal diet to make up the 1.6 per cent filtered lipocaic diet. Portions of the Berkfeld filtrate were stored in the refrigerator and used for parenteral administration. The basal diet was mixed with distilled water in the proportion of 20 cc. of water to 100 grams of diet to make it similar in consistency to the lipocaic diets. The 1.6 per cent inositol diet was prepared by substituting the appropriate amount of inositol² for an equivalent amount of glucose in the basal diet. At the end of the 20th day of the experi-

² Prepared from tablets of inositol—Winthrop Chemical Company—H 1.4413 1z.

mental period, the animals were killed and total lipids in each whole liver determined by methods previously described (10).

RESULTS. The results are summarized in table 1.

The 6 rats on the basal diet were found to have an average of 28.76 per cent of fat in the livers after 20 days. Five rats fed the 1.6 per cent inositol diet consumed an average of 164.0 mgm. of inositol daily. The livers of these animals contained an average of 30.16 per cent total lipids, which does not differ significantly from those on the basal diet. In sharp contrast to these, the three groups of rats given the basal diet plus pancreas extract showed greatly reduced amounts of liver fat. The unfiltered solution of lipocaic was somewhat more effective than an equal amount passed through the Berkfeld filter, indicating some loss of lipotropic potency from filtration. The 5 rats given the filtered solution of lipocaic subcutaneously showed no ill effects from the daily injections

TABLE 1

A comparison of the effects of oral inositol and of oral and parenteral lipocaic on dietary fatty livers in white male rats

NO. OF ANI- MALS	EXPERI- MENTAL PERIOD	MEDICATION PER RAT PER DAY	AVERAGE WT. OF RATS	AVERAGE GAIN IN WT. PER RAT	TOTAL LIVER LIPIDS	STANDARD ERROR
	<i>days</i>	<i>mgm.</i>	<i>grams</i>	<i>grams</i>	<i>grams per cent</i>	
6	20	None	152.8	8.5	28.76	± 3.597
5	21	164.0 mgm. inositol	116.4	9.8	30.16	± 3.389
6	20	140 mgm. unfiltered pancreas ex- tract	151.8	15.8	5.06	± 0.449
6	20	136 mgm. filtered pancreas ex- tract	150.2	12.5	7.37	± 0.592
5	20	160 mgm. filtered pancreas ex- tract given subcutaneously	150.4	11.8	5.08	± 0.120

and the marked reduction of liver fats in these animals indicated that lipocaic is effective on parenteral administration.

DISCUSSION. The failure of inositol to prevent the development of dietary fatty livers in white rats in these experiments under conditions where the same amount of pancreas extract containing lipocaic was entirely effective indicates that inositol cannot be the substance in pancreas which accounts for its lipotropic potency in these animals. The apparent disagreement of these results with those of Gavin and McHenry and of Forbes may be due to several factors. First, our diet contained a high per cent of fat and all B vitamins and therefore differed from work done previously on the effects of lipocaic and inositol on fatty livers in rats. Second, our animals were normal at the beginning of the experiment, had not been depleted of B vitamins and fat and therefore may have reacted differently to the test diets; and third, the relative amounts of the various members of the B complex contained in brewer's yeast differ considerably from those used by McHenry and Forbes.

As noted above, the extract containing lipocaic used in these experiments was

prepared by extracting fresh beef pancreas glands with 95 per cent ethyl alcohol acidified with sulfuric acid to pH 3. It was defatted by chilling, adjusted to pH 7.0 with calcium oxide, and the precipitated sulphate removed by filtration. Such extracts contain almost the entire activity of the fresh raw pancreas in the sense that the material obtained from 100 grams of pancreas exerts just about the same effect as the pancreas itself when fed. In this connection, it is necessary to call attention to an error in the report of Entenman, Montgomery and Chaikoff (11) which led them to conclude that the administration of lipocaic equivalent to 100 grams of pancreas to completely depancreatized dogs failed to prevent the fall of cholesterol, phospholipids or total fatty acids of the blood below preoperative levels and also failed to prevent fatty livers. These workers used lipocaic preparations no. H-7240, H-7423 and H-7104A, which they obtained from the Eli Lilly Company. Entenman and his associates mistakenly assumed that these extracts were prepared from fresh pancreas by the method of Dragstedt, Prohaska and Harms and so erred in their calculations. As a matter of fact the extracts used by Entenman and his associates were prepared from various residues discarded in the usual manufacture of insulin. Since these extracts were at our request freely distributed by the Eli Lilly Company for both laboratory and clinical studies, it may be useful to record the method used for their production.

Lipocaic fractions no. H-7240, H-7423, and H-7104A were prepared by the Eli Lilly Company in co-operation with this laboratory where the assays were carried out on depancreatized dogs by methods previously described (12).

In the preparation of fraction H-7240, the pancreas residue remaining after the extraction of insulin was extracted twice with 40 per cent alcohol at pH 9. The filtrate was adjusted to pH 5.5 with sulfuric acid and concentrated to a syrup. During concentration, the pH was adjusted with NaOH to maintain a value between 5.5 and 7.0. The syrup was dried in vacuo, defatted with naphtha and redried in vacuo. Two and six-tenths grams were obtained from 100 grams of original pancreas. This fraction was assayed on 12 depancreatized dogs in doses ranging from 2.5 to 10 grams daily. In several of the experiments, a dose of 2.5 grams daily seemed to be effective and in all experiments, doses of 4 grams daily and above proved to be effective and curative. In their experiments, Entenman and his associates found that a daily dose of 0.35 gram of this preparation was ineffective, but that a daily dose of 2.54 grams was effective.

In the preparation of fraction H-7423, the pancreas residue remaining after the extraction of insulin was re-extracted with 40 per cent alcohol and CaO added to give a pH of 10. The mixture was filtered and the residue re-extracted. The combined filtrates were brought to a pH of 6.5 to 7 with sulfuric acid, the solution chilled and filtered. The filtrate was reduced to a very small volume just short of a syrup and precipitated with 8 volumes of absolute alcohol. The precipitate was dried in vacuo. From 100 grams of original pancreas, 0.7 gram of dried material was obtained.

This preparation was found effective in relieving the fatty livers in depan-

creatized dogs in a daily dose of from 3 to 5 grams depending somewhat on individual variations in different animals and on the amount of fat in the diet. It is clear that this represents a good deal more than 100 grams of original pancreas. It should be emphasized, however, that the pancreas had first been extracted with acid alcohol and accordingly a good deal of the original lipocaic already removed, and furthermore, that the extraction by this method does not remove all of the lipocaic remaining in the pancreas residue. Entenman and his associates gave as a daily dose 0.5 gram of this extract and found that it was ineffective. This is in agreement with our data. It is incorrect, however, to state that this represents the amount of lipocaic that is derived from 100 grams of raw pancreas. It is rather the amount of lipocaic that it was possible to extract from the residue remaining from 100 grams of raw pancreas that had first been extracted with acid alcohol in the preparation of insulin.

In the preparation of insulin the acid-alcohol extract of the whole pancreas may be partly evaporated to drive off the alcohol and the remaining acid-water solution concentrated by evaporation and chilled and the fat skimmed off. The addition of a mixture of strong alcohol and ether to this acid-water phase results in the formation of a precipitate. This, when dried, was found to be effective in a dose of 0.75 to 1.5 grams per day indicating that much lipocaic is removed from pancreas in the acid alcohol extraction for the preparation of insulin. Fraction H-7104A employed by Entenman and his associates was prepared as follows: In the acid-water phase of the insulin procedure referred to above, the addition of alcohol to yield an ultimate concentration of 80 per cent results in the formation of a precipitate relatively free from insulin. This preparation was found to contain an effective dose of lipocaic, in from 2 to 3 grams of dried substance. From 100 grams of original whole pancreas, 0.37 gram of this material was obtained. It is thus apparent that much of the lipocaic is lost in various stages of the insulin procedure, in addition to that remaining behind in the original gland residue. Entenman and his associates employed this fraction in a daily dose of 0.37 gram and for the most part found it ineffective. This is likewise in agreement with our own data but it should not be assumed that this is the amount of lipocaic which could be secured from 100 grams of original raw pancreas.

The finding that pancreas extracts containing lipocaic exert their characteristic lipotropic effect when given by subcutaneous injection is important from the standpoint of the physiology of this substance. It indicates that the active principle in pancreas does not need to pass into the alimentary tract to become active. The extremely small standard error in the groups of animals given lipocaic parenterally suggests that this may be a more efficient method of administration and accordingly, particularly useful for assay studies.

CONCLUSIONS

1. Inositol in doses of 164 mgm. per day fails to prevent marked deposition of fat in the liver of the white rat fed a high fat, low protein diet containing 5 per cent brewer's yeast. Defatted alcoholic extracts of beef pancreas contain-

ing lipocaic exert a marked lipotropic effect when fed in the same dose. Inositol is thus not the active principle in beef pancreas which prevents this type of dietary fatty liver in rats.

2. Pancreatic extracts sterilized by Berkefeld filtration exert just as marked a lipotropic effect when given by subcutaneous injection as when fed.

REFERENCES

- (1) HERSHEY, J. M. *This Journal* **93**: 657, 1930.
- (2) MCHENRY, E. W. AND J. M. PATTERSON. *Physiol. Reviews* **24**: 128, 1944.
- (3) DRAGSTEDT, L. R., V. J. PROHASKA AND H. P. HARMS. *This Journal* **117**: 175, 1936.
- (4) BEST, C. H. AND C. C. LUCAS. *Vitamins and hormones*. Vol. I., New York, Academic Press, 1943.
- (5) MCHENRY, E. W. AND G. GAVIN. *Science* **91**: 171, 1940.
- (6) GAVIN, G. AND E. W. MCHENRY. *J. Biol. Chem.* **141**: 619, 1941.
- (7) GAVIN, G. AND E. W. MCHENRY. *J. Biol. Chem.* **139**: 485, 1941.
- (8) FORBES, J. C. *Proc. Soc. Exper. Biol. and Med.* **54**: 89, 1943.
- (9) OWENS, F. M., J. G. ALLEN, D. STRINGER AND L. R. DRAGSTEDT. *Federation Proc.* **1**: 65, 1942.
- (10) CLARK, D. E., M. L. EILERT AND L. R. DRAGSTEDT. *This Journal* **144**: 620, 1945.
- (11) ENTENMAN, C., M. L. MONTGOMERY AND I. L. CHAIKOFF. *This Journal* **141**: 221, 1944.
- (12) DRAGSTEDT, L. R., C. VERMEULEN, W. C. GOODPASTURE, P. B. DONOVAN AND W. A. GEER. *Arch. Int. Med.* **64**: 1017, 1939.

FORMULATION OF THE PRINCIPAL FACTORS AFFECTING THE RATE OF UPTAKE OF CARBON MONOXIDE BY MAN¹

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The rate of uptake of carbon monoxide in ambient air by the blood of man has been studied for a variety of purposes from the elucidation of the mechanism of gas transfer across the alveolar wall (1) to the design of ventilation systems for vehicular tunnels (2). The subject has been reviewed extensively (3, 4, 5, 6), and the trend from an empirical expression of the phenomenon to a theoretical treatment is clearly marked. The recent comprehensive study of Forbes, Sargent and Roughton (6) has done much toward the clarification of the factors responsible for the determination of the rate of uptake of CO.

Early in 1943 the need arose in the naval service for a reliable means of predicting as a function of time the degree of saturation with CO of the blood hemoglobin of personnel exposed to concentrations of CO in air under various conditions of physical activity. Studies in this laboratory resulted in a simple formulation of the major factors affecting the rate of uptake of CO, and a preliminary equation was presented for limited distribution in a Navy report (7). Since that time the equation has been used in a variety of applications, both aboard ship and in aircraft, and aside from a few minor modifications it has been found to be adequate for the purpose intended. The data on which the derivation was based, together with the derivation itself, are presented in this paper.

METHODS. The 32 subjects used in these experiments were volunteer male naval personnel ranging in age between 18 and 40 years. Mixtures of carbon monoxide in air were inspired through a rubber mouthpiece and two-way valve arrangement from a 600 liter Tissot type gasometer so that the total volume of respiration could be recorded. Pure CO was generated by the interaction of sulfuric and formic acids, and was mixed with air in the gasometer to give the desired concentration. The concentration of each batch of gas was checked by the iodine pentoxide method of CO analysis (8). Concentrations of CO in air ranging from 0.9 to 21.8 parts per 10,000 were used. Venous blood samples from the antecubital vein were obtained from each subject immediately prior to the exposure, during the exposure, and at the end of the exposure. The blood carboxyhemoglobin (COHb) was determined as volumes per cent CO by the method of Horvath and Roughton (9), and conversion to per cent COHb was made by multiplying the volume per cent values by five. Subjects were studied sitting quietly at rest, both at normal ambient barometric pressure and at reduced barometric pressure, 520 mm. of mercury, in a low pressure chamber.

¹ The material in this article should be construed only as the personal opinions of the writers and not as representing the opinion of the Navy Department officially.

Studies were also carried out on subjects walking on a level treadmill at 2.7 miles per hour. Arrangements were made in some experiments to collect the

TABLE 1

Summarized data on the uptake of CO by the blood of normal men

SUBJECT	AIR CO CONC.	EX- POSURE TIME	MINUTE VOLUME	BLOOD VOLUME	INITIAL BLOOD COHb	FINAL BLOOD COHb	EXPTL. Δ % COHb	CALCU- LATED Δ % COHb	DEVIATION OF CALCULATED FROM EXPTL. Δ % COHb
	<i>parts/ 10,000</i>	<i>minutes</i>	<i>liters</i>	<i>liters</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
MAR.....	20.0	20	9.6	6.18	2.2	14.5	12.3	13.4	+1.1
COV.....	20.0	20	6.6	5.61	1.6	11.2	9.6	10.0	+0.4
KLI.....	20.0	20	6.1	5.76	0.0	10.7	10.7	9.0	-1.7
HOL.....	20.0	20	5.8	4.98	4.0	13.0	9.0	10.0	+1.0
BLA.....	20.0	20	17.9	5.37	0.4	34.6	34.2	28.7	-5.5
KRU.....	20.0	20	22.4	6.03	1.3	32.3	31.0	32.0	+1.0
TUS.....	20.0	20	24.2	6.09	3.3	34.9	31.6	34.1	+2.5
MCB.....	20.0	20	22.8	6.24	2.9	33.7	30.8	31.4	+0.6
SCE.....	20.0	20	19.1	5.49	3.6	34.9	31.3	29.8	-1.5
BIT.....	15.0	24	30.1	7.08	1.7	35.8	34.1	32.9	-1.2
SCA.....	15.0	20	23.5	5.49	3.7	28.9	25.2	27.6	+2.4
DEM.....	10.0	30	17.6	5.22	6.9	27.0	20.1	21.8	+1.7
DIE.....	10.0	30	18.9	5.49	2.4	26.1	23.7	22.2	-1.5
ERI.....	10.0	30	17.1	5.16	5.0	24.8	19.8	21.3	+1.5
PIT.....	17.2	15	18.4	6.09	3.3	23.0	19.7	16.8	-2.9
FED.....	18.7	15	22.3	5.85	7.2	26.5	19.3	23.0	+3.7
WAG.....	21.8	15	18.7	5.28	4.2	33.8	29.6	25.0	-4.6
SHA.....	14.2	20	24.9	6.39	6.4	26.1	19.7	23.8	+4.1
CAT.....	14.1	20	17.8	5.67	5.4	26.1	20.7	19.0	-1.7
JAM.....	12.9	20	21.1	5.28	0.8	24.8	24.0	22.2	-1.8
WIS.....	9.0	30	17.5	5.04	2.8	24.7	21.9	20.2	-1.7
LEG.....	9.0	30	18.5	5.64	8.9	23.6	14.7	19.0	+4.3
HIN.....	9.4	30	18.1	5.28	8.9	29.5	20.6	20.7	+0.1
SPE.....	5.5	45	15.6	5.04	3.6	19.3	15.7	16.5	+0.8
WAR.....	5.6	45	17.4	4.83	3.0	25.4	22.4	19.5	-2.9
REI.....	5.7	39	20.2	5.61	1.8	20.0	18.2	17.2	-1.0
WAT.....	9.2	30	9.4	5.31	1.8	12.1	10.3	10.6	+0.3
SAW*	0.9	240	5.7	5.22	0.0	6.3	6.3	5.1	-1.2
SCH*	0.9	180	5.2	5.61	3.3	7.8	4.5	3.2	-1.3
PEC*	0.9	270	5.7	5.76	0.4	7.3	6.9	5.2	-1.7
AUD*	1.8	300	5.6	5.55	5.8	16.5	10.7	11.7	+1.0
HAY*	1.8	300	4.7	5.01	3.5	15.0	11.5	10.9	-0.6
N = 32	Mean.....			5.58	3.4				
	S.D.....			0.47	2.4				

* Subjects tested at a simulated altitude of 10,000 feet.

expired air for CO analysis by the iodine pentoxide method. No attempt was made to control smoking before the tests.

RESULTS AND DISCUSSION. The data obtained on the 32 subjects are presented in table 1. The values for blood volume were estimated by multiplying the body

surface area of each individual by the factor, 3.0 liters of blood per square meter of body surface. Both Rowntree and Brown (10) and Gibson and Evans (11) have shown that blood volume and body surface area are in constant ratio in adult males, and the ratio from the data of the latter investigators has been used here. The initial concentration of CO in the blood immediately before the test was found to vary from 0.00 to 1.78 per cent COHb. The individual values for smokers were consistently higher than those for non-smokers, and some correlation was seen to exist between the degree of the smoking habit and the blood level of COHb. The mean for the group was calculated to be 3.44 per cent COHb, standard deviation ± 2.35 per cent. Because of this relatively large variability in the starting concentration of COHb, the increase in concentration of COHb as a result of the test exposure to CO was expressed as the difference, Δ per cent COHb, between the initial and final blood COHb concentrations.

From *a priori* reasoning it was evident that the amount of COHb formed in the blood per unit time was a function of the amount of CO available in the lungs during that time. In order to simplify the reasoning it was assumed that the events determining the rate of uptake of CO occur in four areas, namely, the ambient air, the alveolar air, the alveolar and lung capillary membranes, and the blood. In a system composed of only the first two areas the periodic flushing of the alveoli with ambient air would cause the composition of alveolar air to approach that of ambient air, and an increase in rate or depth of respiration would hasten this approach. Hence, it may be said that an increase in minute volume of respiration ($\text{rate} \times \text{depth}$) minimizes the difference between alveolar air and ambient air. In the system under consideration, however, a constant loss of CO occurs from the alveolar air by diffusion through the alveolar walls into the blood, so that a concentration differential is maintained between ambient CO and alveolar CO. In this case an increase in minute volume may be regarded as increasing the mean alveolar CO concentration. An increase in minute volume also increases the total quantity of air brought into contact with the alveoli per unit time, thereby making a greater quantity of CO available for diffusion during one inspiration, so that the alveolar CO concentration is decreased to a lesser extent per unit of CO uptake by the blood. Thus since an increase in minute volume tends to increase the mean alveolar CO concentration in two ways, the rate of saturation of the blood with CO may be assumed to be directly proportional to the minute volume of respiration.

In any single respiratory cycle of inspiration and expiration the alveolar CO concentration rises and falls; however, in a sufficiently long unit of time, e.g., one minute, and provided the minute volume is constant, the alveolar CO concentration may be considered to be a constant mean value directly proportional to the ambient CO concentration.

From the observations of Krogh (1) the permeability of CO through the alveolar wall in a given individual is constant in the range from normal alveolar volumes upward toward volumes approaching the vital capacity. Thus for any given individual, changes in permeability with changing minute volume are not a factor in the rate of CO uptake. The permeability of the alveolar wall

for CO apparently does vary significantly among individuals and is undoubtedly partially responsible for individual differences in the rate of CO uptake, as pointed out by Forbes, Sargent and Roughton (6). These differences are small relative to the influence of other factors, so the effect of permeability differences was not considered in the present study.

As the uptake of CO progresses, the concentration of COHb in the blood increases. The total volume of blood, and hence hemoglobin, available will be a determining factor in the rate of rise of the blood COHb concentration and this rate can be considered to be inversely proportional to the blood volume.

A factor in the rate of uptake of inert gases such as nitrogen is that as the uptake progresses the partial pressure of the gas in the blood increases with a resulting increase in back diffusion across the alveolar wall. Thus the rate of uptake is dependent on the concentration of the gas in the blood. In the case of CO this effect is apparently negligible for concentrations of COHb up to one-third of the equilibrium value with the air CO concentration. This may be seen in figure 1 where the increase in per cent COHb with time has been plotted for 5 of the 32 subjects studied in the present experiments. The uptake is essentially a straight line over the range of COHb studied, and re-examination of the data of other investigators who have made similar measurements (12, 13) substantiates this observation. The reason for the independence of the rate of uptake of CO from blood concentration in the lower ranges of COHb saturation is the fact that CO is bound tightly to hemoglobin and the partial pressure of CO in equilibrium with low percentages of COHb is small. This is apparently true even of exposures to very low concentrations of CO in the ambient air, *circa* one to three parts per 10,000, in which case the uptake is linear up to values of COHb of approximately one-third the equilibrium value (14). For higher ranges of saturation the scanty and unsatisfactory nature of existing data on COHb concentrations approaching equilibrium make it impractical at this time to attempt a quantitative expression of the entire uptake curve.

With these considerations in mind, and as appreciated early by Haldane (12), it appeared that the total quantity of CO appearing in the blood in the form of COHb during the course of an exposure to air containing CO could be expressed in the form of a constant fraction of the total CO passing into the respiratory system during the exposure thus:

$$(1) \quad \frac{\text{Total blood CO}}{\text{Total CO inspired}} = k$$

Equation (1) can then be broken down as follows:

$$(2) \quad \frac{\text{Blood CO concentration} \times \text{Blood volume}}{\text{Air CO concentration} \times \text{Total respiratory volume}} = k$$

Equation (2) may be written in the form of the equation for a straight line, $y = bx$, thus:

$$(3) \quad \text{Blood CO Conc.} \times \text{Blood Vol.} = k (\text{Air CO Conc.} \times \text{Total Resp. Vol.})$$

The experimental data given in table 1 can then be used, in the proper units, to

test the validity of equation (3) and to establish the value for k . This was done by expressing Δ per cent COHb as cubic centimeters CO per liter of blood (Δ per cent COHb $\times 2$), and parts CO per 10,000 of air as cubic centimeters CO per liter of air (parts CO per 10,000 $\times 0.1$). Both blood volume and total respiratory volume were expressed in liters as given in table 1. The data were plotted in figure 2 according to equation (3), expressing the product of cubic centimeters CO per liter of blood and liters blood volume along the y axis and the product of cubic centimeters CO per liter of air and liters total respiratory volume along the x axis. Thus the values plotted represent total cubic centimeters of CO in the blood versus total cubic centimeters of CO in the inspired air, and the slope of the best line through the data is represented by k . Assuming the line of pass

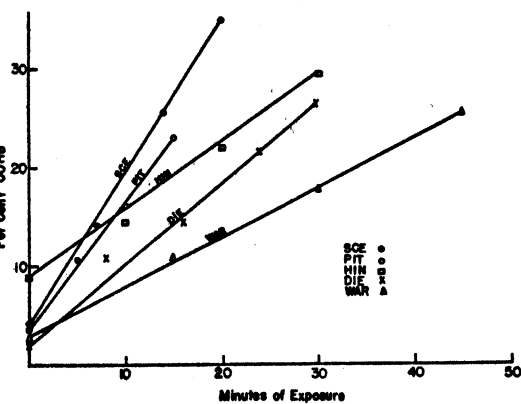


Fig. 1

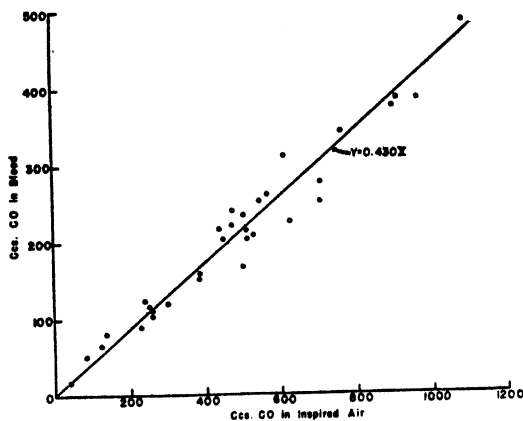


Fig. 2

Fig. 1. Increase in blood concentration of COHb in five normal men during the course of exposure to various concentrations of CO in air.

Fig. 2. Relationship of the amount of CO taken up by the blood to the amount of CO passed through the lungs. Thirty-two subjects, both at rest and engaged in moderate physical activity, and breathing various concentrations of CO in air.

through the origin, a least squares fit to the data yielded a value of 0.430 for k , and a correlation coefficient of 0.97 was calculated indicating the high order of correspondence of the two variables over a wide range of conditions.

Putting the terms in equation (3) in the form of the variables measured in the present study and recombining the terms it is possible to write:

$$(4) \quad \Delta \text{ per cent COHb} = \frac{0.430 \times \text{Parts CO} \times \text{Min. Vol.} \times \text{Exposure Time}}{20 \times \text{Blood Vol.}}$$

where Δ per cent COHb is the total increase of blood COHb as a result of the exposure, Parts CO is the concentration of CO in parts per 10,000 of the ambient air, Min. Vol. is the minute volume of respiration in liters per minute corrected to standard temperature and pressure so as to take into account changes in partial pressure of the CO; Exposure Time is the duration of the exposure in minutes; Blood Vol., is the blood volume in liters (obtained here as square meters body sur-

face area $\times 3.0$), the value 0.430 corresponds to k in equation (3), and the value 20 is the conversion factor for the specific units in equation (4). Combining the constants, equation (4) may be written:

$$(5) \quad \Delta \text{ per cent COHb} = \frac{\text{Parts CO} \times \text{Min. Vol.} \times \text{Exposure Time}}{46.5 \times \text{Blood Vol.}}$$

The values for Δ per cent COHb calculated from equation (5) are included in table 1 for comparison with the values obtained experimentally, and the standard deviation of the error of estimate was found to be 2.3 per cent COHb. Good agreement with the data of previous investigators as summarized in (6) was also obtained when the expected value of Δ per cent COHb was calculated.

The variability of the data as shown in figure 2 is probably due in part to physiological variables such as individual differences in the permeability of the

TABLE 2
Fraction of CO removed from inspired air by the blood

SUBJECT	EXPIRED AIR CO	INSPIRED AIR CO	TOTAL VENTI- LATION	TOTAL CO INSPIRED	BLOOD CO IN- CREASE	BLOOD VOLUME	TOTAL BLOOD CO	CO AB- SORBED BY BLOOD	CO REMOVED FROM INSPIRED AIR
	cc./liter	cc./liter	liters	cc.	cc./liter	liters	cc.	per cent	per cent
PIT.....	0.85	1.72	276.0	475	39.4	6.09	240	50.5	50.6
WED.....	1.10	1.87	334.5	626	38.6	5.85	229	36.6	41.2
WAG.....	1.25	2.18	281.0	613	59.2	5.28	313	51.1	42.7
SHA.....	0.84	1.42	497.0	706	39.4	6.39	252	35.7	40.8
CAT.....	0.76	1.41	356.2	502	41.4	5.67	235	46.8	46.1
JAM.....	0.77	1.29	422.0	544	48.0	5.28	253	46.5	40.3
WIS.....	0.56	0.90	525.8	473	43.8	5.04	221	46.7	37.8
LEG.....	0.58	0.90	555.0	500	29.4	5.64	166	33.2	35.6
HIN.....	0.54	0.94	541.5	509	41.2	5.28	218	42.8	42.6
SPE.....	0.31	0.55	702.5	386	31.4	5.04	158	40.9	43.6
WAR.....	0.34	0.56	783.0	438	44.8	4.83	216	49.3	39.3
REI.....	0.38	0.57	787.0	449	36.4	5.61	204	45.4	33.3
N = 12	Mean.....							43.8	41.2
	S.D.....							5.8	4.4

alveolar wall for CO and in the ratio of tidal air to dead space which are discussed in (6). Furthermore, some variability is to be expected from the individual variation in blood hemoglobin content, which can be corrected for but was not in the present study. The calculation rather than the actual measurement of the blood volume also contributes some error, and finally the uncertainty in the analytical methods used also contribute in part. However, by the use of equation (5) it is possible to predict the rate of uptake of CO for individuals with reasonable accuracy, and it has proven useful in a number of practical applications. There are obviously limits to its use; for example, as the equilibrium value for COHb at a given pressure of CO is approached, the rate of uptake is no longer constant. In general, the equation may be considered valid for values of COHb up to one-third the equilibrium value for the CO concentration under consideration.

From equation (1) it may be seen that $k \times 100$ is the per cent CO absorbed by the blood from the inspired air, and by using the proper units in equation (2) the per cent CO absorbed by the blood may be calculated from the data at hand. This is done in table 2 for twelve of the subjects. The concentration of CO in the expired air was also determined for these subjects and is included in the table, together with the computation of the actual CO absorbed. Comparison of these values for each subject shows good agreement except in a few cases. The means for the two methods agree well with each other and with the least squares value of $k \times 100$ of 43.0 obtained for all the subjects.

The values of the per cent CO absorbed from the inspired air are somewhat lower than those observed by most other investigators (6, 12). The data presented here were all obtained on men engaged in moderate physical activity (mean minute volume of respiration of 19.2 liters S.T.P., per minute), and it is possible that at this rate of ventilation a lower proportion of CO is removed from the inspired air than at rest, because each breath remains in the lungs less time at higher rates of respiration. Veale (15) however has shown that this effect is quite small, even at rates of ventilation up to 48 liters per minute, and his values for the proportion of CO absorbed are consistent with those observed in the present study. Furthermore, calculation by means of equation (2) of the per cent CO absorbed by the blood of the resting subjects reveals no significant difference from the values given in table 2. No explanation of the discrepancy can be given at this time, except that it might be accounted for by differences in analytical technic.

In order to test the possibility that changes in the ambient barometric pressure might grossly affect the rate of uptake of CO, five of the subjects were studied in the low pressure chamber at a total pressure of 520 mm. of mercury breathing air containing 0.9 to 1.8 parts CO per 10,000. As shown by the last five subjects listed in table 1, the uptake of CO was predictable from equation (3) for men breathing air containing CO at altitudes up to 10,000 feet. Although it has clearly been shown (6) that changes in partial pressure of oxygen affect the rate of uptake of CO, this effect is apparently slight in going from sea level to 10,000 feet altitude provided the change in partial pressure of CO is compensated for by correcting the minute volume of respiration to standard temperature and pressure.

SUMMARY

The rate of uptake of carbon monoxide has been shown to be constant with respect to blood concentration of COHb, up to values of one-third the equilibrium level, when air containing CO in the range 1 part to 20 parts per 10,000 is breathed by men at rest or engaged in moderate physical activity.

An equation has been derived which serves as a means of estimating in man the degree of blood saturation with CO as a result of exposure to air containing this gas as follows:

$$\Delta \text{ per cent COHb} = \frac{\text{Parts CO} \times \text{Minute Volume} \times \text{Exposure Time}}{46.5 \times \text{Blood Volume}}$$

The equation is valid for values of per cent COHb up to one-third the equilibrium value for the air concentration of CO under consideration. Within this range Δ per cent COHb may be estimated within a degree of error whose standard deviation is ± 2.3 per cent COHb.

The fraction of CO removed from the inspired air by the blood was found to be constant as uptake progressed, and the mean for a group of twelve men was 41.2 per cent with a standard deviation of ± 4.4 per cent. This value is somewhat lower than that observed by previous investigators.

REFERENCES

- (1) KROGH, M. *J. Physiol.* **49**: 271, 1915.
- (2) HENDERSON, Y., H. W. HAGGARD, M. C. TEAGUE, A. L. PRINCE AND R. M. WUNDERLICH. *J. Ind. Hyg.* **3**: 79, 1921.
- (3) DRINKER, C. K. Carbon monoxide asphyxia. Oxford University Press, 1938.
- (4) KILLICK, E. M. *Physiol. Rev.* **20**: 313, 1940.
- (5) VON OETTINGEN, W. F. U. S. Public Health Service. Public Health Bull. no. 290, 1944.
- (6) FORBES, W. H., F. SARGENT AND F. J. W. ROUGHTON. *This Journal* **143**: 594, 1945.
- (7) BEHNKE, A. R., W. A. WHITE, W. V. CONSOLAZIO AND N. PACE. U. S. Navy Bureau of Medicine and Surgery Research Project X-160, Report no. 2, 1943.
- (8) CONSOLAZIO, W. V., L. J. PECORA, E. MACDONALD, H. A. COLLISON, H. L. KREUGER, W. T. PLATT, J. D. O'NEAL AND J. A. RAE. U. S. Navy Bureau of Medicine and Surgery Research Project X-417, Report no. 5, 1945.
- (9) HORVATH, S. M. AND F. J. W. ROUGHTON. *J. Biol. Chem.* **144**: 747, 1942.
- (10) ROWNTREE, L. G. AND G. E. BROWN. The volume of the blood and plasma. W. B. Saunders Co., 1929.
- (11) GIBSON, J. G. AND W. A. EVANS. *J. Clin. Investigation* **16**: 317, 1937.
- (12) HALDANE, J. *J. Physiol.* **18**: 430, 1895.
- (13) SAYERS, R. R., W. P. YANT, E. LEVY AND W. B. FULTON. U. S. Public Health Service. Public Health Bull. no. 186, 1929.
- (14) PITTS, G. C. U. S. Navy Bureau of Medicine and Surgery Research Project X-417, Report no. 6, 1945.
- (15) VEALE, A. P. *Colliery Guardian* **123**: 1541, 1922.

PERMEABILITY OF THE PLACENTA OF THE GUINEA PIG TO INORGANIC PHOSPHATE AND ITS RELATION TO FETAL GROWTH

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Our previous studies of the placenta have been made with the concept of comparative physiology in mind; it has been our purpose, using radioactive sodium as the test substance, to measure differences in permeability of the four morphological types of placentas and to relate our findings to their histological structure. It is our purpose now, making further use of isotopic material, to describe the permeability of a single type of placenta with respect to the physico-chemical properties of ionic and molecular species. It is evident that such a study will increase our understanding of some of the problems of fetal nutrition. We have chosen the guinea pig for these experiments because its placenta belongs to the same morphological group, the hemochorial, as that of man and because of its favorable gestation period (67 days) which readily permits separation of different stages of gestation.

The present experiments have as their objective the evaluation of the permeability of the placenta to inorganic phosphorus, using radioactive phosphorus (P^{32}) as the tracer substance. The fundamental assumption in this use of a radioactive isotope is that the radioactive substance crosses a membrane precisely as does its naturally occurring isotope, and that, consequently, the two isotopes cross the membrane in the same proportion as is found in the fluid bathing the membrane. In the case of the placenta this means that

$$\frac{P_{mp}^{32}}{P_{mp}} = \frac{P_f^{32}}{P_f}, \quad (1)$$

where P_{mp}^{32} refers to the concentration of radioactive isotope present as inorganic phosphate in the maternal plasma; P_{mp} , to the concentration of phosphorus of normally occurring inorganic phosphate in the maternal plasma; and P_f^{32} and P_f are the quantities of the corresponding isotopes transferred to the fetus during the course of an experiment.

After the injection of radioactive phosphate into a maternal vein, its concentration, P_{mp}^{32} , in the plasma rapidly diminishes due to its loss to the extra-cellular fluid and to the tissue cells of the mother. Only its average value measured over the observed period of transfer is suitable for substitution in equation 1. Our first series of experiments, therefore, was designed to measure the time-concentration curve of radioactive phosphate after its injection and from this curve the average concentration was obtained.

Equation 1, moreover, is valid only if none of the radioactive isotope trans-

ferred to the fetus is returned or lost from fetus to mother during the period of observation. It is reasonable to assume that early after introduction of the radioactive isotope into the maternal circulation, its concentration in fetal plasma remains so low compared to that of maternal plasma that only a negligible quantity returns from fetus to mother. We take this condition to be satisfied during that period of transfer when the concentration of radioactive isotope in the fetus, referred to an average concentration of one beta particle per cubic centimeter of maternal plasma, increases linearly with time. A series of experiments consequently was designed to estimate the length of the period of linear accumulation in the fetus.

With the information derived from these two sets of observations we have next proceeded to measure the rate of transfer of inorganic phosphate to fetuses of various gestation ages and from these data to calculate the permeability of the placenta to phosphate and to estimate the supply of inorganic phosphate to the fetus as this is related to the requirements of fetal growth.

METHODS. Radioactive phosphorus (P^{32}) was prepared in the 60-inch cyclotron of the Department of Terrestrial Magnetism, Carnegie Institution of Washington. The phosphorus was separated from the copper of the target by precipitation as ammonium phosphomolybdate. This precipitate was dissolved and the phosphorus then reprecipitated as magnesium ammonium phosphate and from this sodium hydrogen phosphate, pH 7.4, was prepared. The preparation was examined for possible contaminating radioactivities by measuring the rate of radioactive decay. No half-life values were found other than that characteristic for P^{32} .

Experiments were performed to follow changes with time in the concentration of P^{32} in blood plasma after its injection as radioactive phosphate into the vein of a foreleg of 6 male guinea pigs under nembutal anesthesia. Samples of blood of about 1 cc. were collected from a carotid cannula into ice cold syringes (1); the blood was centrifuged immediately after collection and measurements made of the concentration of radioactive phosphorus in the plasma.

The placental transfer was studied in eight pregnant guinea pigs each at a different stage of gestation. The experimental procedure with these animals followed closely that described for radioactive sodium (2). After an appropriate interval of time following intravenous injection of the radioactive salt into the pregnant mother, the fetuses were delivered by abdominal hysterotomy and immediately thereafter a sample of blood was taken from the mother's heart and promptly centrifuged. The fetuses and placentas were weighed; the fetuses were then minced and dried overnight in an oven at 110°C. An aliquot of the ground fetal remains was then measured for radioactivity; in 5 litters, 2 grams of dry residue were measured; in the others 3 grams were measured. The radioactivity was measured by a temperature controlled, pressure ionization chamber connected to a string electrometer (2); these measurements were corrected for self-absorption of radiation by the sample. The correction curve for self-absorption by liquid samples was established by measuring the radiation of a dry sample of radioactive salt and then observing the reduction in measurable

radiation which followed addition of water or blood plasma to the soluble salt. As is to be seen in figure 1 the absorption of radiation by equal volumes of water and plasma was found to be practically equal. The correction curve for absorption of the dried and ground fetal remains (fig. 1) was established by injecting a sample of sodium phosphate of known radioactivity into a fetus either intraperitoneally or by way of an umbilical vein, grinding the dried fetal remains until homogeneous by test with respect to radioactivity and then measuring the radioactivity of increasing amounts of this dried material.

The concentration of inorganic phosphorus in the plasma of two pregnant animals was determined by the method of Fiske and Subbarow (7) and was found to be 4.42 and 4.43 mgm. per 100 cc.

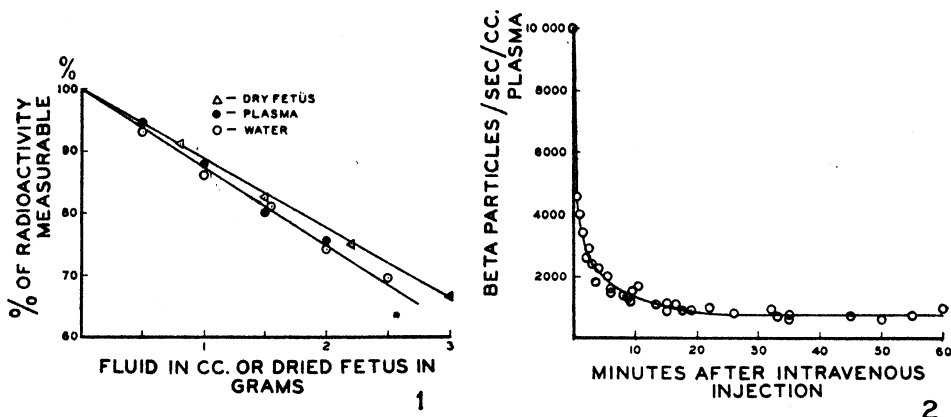


Fig. 1. Absorption of radiation from known quantities of P^{32} by varying amounts of fluid or desiccated fetal tissue. The cup containing the material had a diameter of 5 cm.

Fig. 2. Change in concentration of P^{32} in the plasma with respect to time. The points were adjusted to a standard basis by multiplying them all by a factor which brought the initial concentration to 10,000 beta particles per second per cubic centimeter of plasma, as explained in the text.

RESULTS. *The time concentration curve for P^{32} in plasma after its intravenous injection.* The calculation of the placental transfer rate for phosphate as expressed in equation 1 depends upon an accurate estimation of the average maternal plasma concentration of P^{32} that is maintained during the period of transfer under observation. This entails a precise description of the concentration of P^{32} in maternal plasma from the time of its intravenous injection until the time of delivery of the fetuses. Multiple sampling of maternal blood was avoided by establishing a time-concentration curve in advance of the transfer studies through injection of a known quantity of P^{32} into each of 6 male guinea pigs (fig. 2). The curve was standardized so that concentrations for a given pregnant animal could be deduced from it on determination of a single plasma value at a known time. This was accomplished as follows: For each of the males studied we calculated the concentration in plasma of P^{32} at zero time which would have been present had the injected P^{32} been distributed uniformly in

the plasma of an animal with a standard plasma volume of 4.3 cc. per 100 grams body weight (5). This initial concentration in each animal was multiplied by the necessary factor to convert it to a standard value of 10,000 beta particles per second per cubic centimeter of plasma. Subsequent plasma measurements in a given animal were then multiplied by the same factor. This device was a valid test of the uniformity of the data among the animals (fig. 2). Since the standard plasma volume applied from animal to animal is a constant, the conversion is equivalent to relating all concentrations to a unit quantity of P^{32} per unit body weight.

For this standard curve the average concentration considered to have existed during the observed interval of placental transfer would be the area under the curve as measured by a planimeter for that interval divided by the time of the interval in minutes. The average plasma concentration of a given female could thus be calculated by multiplying the actual concentration of P^{32} in her plasma at the time of delivery of the fetuses by the ratio, derived from the standard curve of figure 2, of the ordinate of average concentration for the period of transfer observed to the ordinate at time corresponding to delivery of the fetuses. Average maternal values thus calculated are listed in the last column of table 1. In all cases the concentration of P^{32} is expressed as beta particles per second per cubic centimeter of plasma.

Equation 1 holds only if all the P^{32} in the maternal plasma exists as inorganic phosphate. In experiments of short duration, such as those reported here, it has been demonstrated that practically no P^{32} combines as the organic form in plasma (3, 4).

The period of linear transfer of P^{32} to the fetus. We have discussed the necessity of demonstrating that early after its injection into the maternal vein the passage of P^{32} is unidirectional across the placenta from mother to fetus with little or no loss of P^{32} from fetus to mother. Since, after injection, the P^{32} in the maternal plasma is continually declining and is therefore entering the fetus at a diminishing rate it is necessary to refer all fetal P^{32} activities to a maternal plasma activity of one beta particle per second per cubic centimeter of plasma as an *average* in time for the period of transfer observed. This is accomplished by dividing the P^{32} per gram of fetus by the average P^{32} per cubic centimeter of maternal plasma that has existed over the period of transfer observed. It is assumed that during the early period after injection over which this ratio increases linearly with time the loss of P^{32} from fetus to mother is negligible.

The early value of P^{32} in the plasma of the fetus, and thus its tendency to return to the mother, would of course depend upon the rate of removal of P^{32} from the plasma to fetal tissue depots in the combined organic form or as bone phosphate. Indeed one might ask whether the calculated transfer rate is not more an expression of the capacity and rate of this recombination process than it is an expression of the rate of transfer through the placental membrane. Thus larger fetuses with larger reservoir depots for the uptake of P^{32} from the plasma might yield faster transfer rates. Or again comparison of rates for different substances might be complicated by the fact that each substance in the

organism has its own peculiar depot or distribution system with its own capacity and rate for exchange with plasma substance. It is our belief that in working

TABLE 1

Values from which the data of figures 3 and 4 have been derived

Delivery time refers to the interval between injection of radioactive phosphate into the mother and delivery of the fetuses

FETAL WEIGHT	DELIVERY TIME	PLACENTAL WEIGHT	TOTAL BETA PARTICLES OF FETUS	BETA PARTICLES IN MATERNAL PLASMA PER SECOND PER CC.	
				Found	Average*
<i>grams</i>	<i>minutes</i>	<i>grams</i>	<i>per second</i>		
3.2	40.3	1.4	282	367	592
2.8	43.0	0.9	218		
2.9	45.0	1.0	248		
33.8	31.5	3.0	2900	326	610
35.1	32.0	3.0	2680		
39.6	32.6	3.7	3840		
34.1	33.3	2.6	1980		
41.7	61.0	4.1	13100	705	1007
43.2	61.5	4.5	17400		
43.0	61.7	4.4	15400		
40.7	33.4	2.8	2430	334	602
41.3	32.8	2.7	3277		
43.9	33.8	3.5	6080		
47.7	32.3	3.1	5220		
52.8	35.3	3.5	3620	263	453
52.8	36.1	3.6	3420		
52.9	37.2	3.3	4160		
63.0	38.9	4.8	6120		
75.1	32.2	4.3	5325	390	703
83.6	33.0	4.5	11900		
86.1	32.6	4.3	11370		
91.4	31.7	4.6	13050		
93.7	30.0	4.3	8500	1210	2260
95.0	30.8	5.0	9360		
105.3	87.0	5.9	41000	552	717

* The average activity in the maternal plasma for the duration of the experiment has been calculated as explained in the text. The single maternal plasma figure applies to an entire litter.

within the linear period of each individual substance we are measuring the transfer rate at a time when the concentration of tagged substance is relatively low in the fetal plasma, and therefore at a time when the calculated transfer rate is a true measure of the limitations of the placental barrier itself. Variations in

the capacity of, and in the rate of uptake by, the depot systems would cause fluctuations in the length of the linear period but not in the intrinsic rate of the placental membrane transfer itself as measured by our method.

To estimate the length of the period of linear transfer, three litters of approximately the same gestation age were delivered 30, 60 and 90 minutes after injection. As seen in figure 3, a linear rate of transfer exists for at least 60 minutes. In view of this finding, the routine procedure for the measurement of placental transfer rates has been to deliver the fetuses between 30 and 45 minutes after injection of P^{32} into the maternal circulation.

The placental transfer rate for phosphate at different gestation ages. The placental transfer rate for phosphate was calculated from equation 1. Values for P_f^{32} and P_{mp}^{32} , the latter averaged for the period of transfer observed, are given in

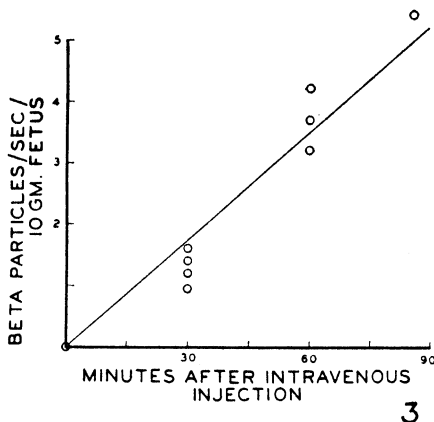


Fig. 3. Accumulation of P^{32} in a unit weight of fetal tissue as a function of time of delivery. The observations on which the curve is based are given in table 1. The observed radioactivity of a unit weight of fetal tissue has in each instance been divided by the average maternal plasma activity for the period of the experiment, and so has been referred to an average plasma activity of one beta particle per second per cubic centimeter.

table 1. P_{mp} was found by measurement to be 0.044 mgm. per cc. of maternal plasma. The value of P_f derived from these data was finally converted to milligrams of inorganic P transferred across one gram of placenta in one hour. The results are presented in figure 4. It is seen that about ten times as much inorganic phosphorus is transferred across a unit weight of placenta near term as across the same weight of the 31-day placenta.

Fetal need for phosphorus relative to the supply across placenta. The ratio of the quantity of a substance supplied to the fetus to the amount of that substance retained by the fetus in its growth has been called the safety factor for that substance (2). The quantity of inorganic phosphorus reaching the fetus per hour across the placenta is calculated as explained above. The increment of phosphorus retained by the fetus during an hour's growth is equal to the fetal weight times the hourly per cent weight increase of the fetus times the total phosphorus in a unit weight of fetal tissue. Total phosphorus of the fetal

tissues was determined by the method of Fiske and Subbarow (7) after dry ashing overnight at 450°C. in a muffle furnace and hydrolyzing the dark ash residue in an oven with 10 per cent HCl. The filtrate from this was shown to contain all the phosphorus in an experiment in which a mouse was injected with radioactive inorganic phosphorus 48 hours before it was sacrificed; after the acid treatment, the filtrate contained all the radioactivity and the ash residue none.

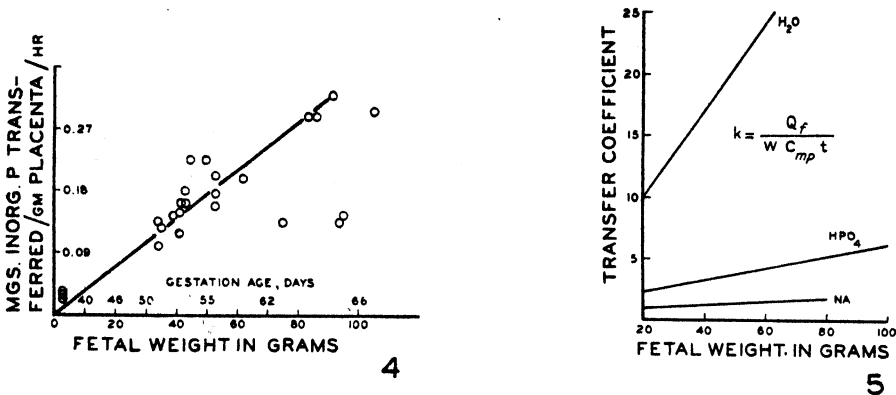


Fig. 4. Variation of the rate of transfer of inorganic phosphorus per unit weight of placenta with respect to fetal weight or gestation age. The points have been derived from the data of table 1. Observations on the two fetuses weighing 95 grams and the one weighing 75 grams have not been considered in fitting the curve; the low values are perhaps due to their being at term (2).

Fig. 5. Transfer coefficients for sodium, inorganic phosphate and water at various fetal weights. The coefficients are defined in the text.

TABLE 2

Fetal need for phosphate relative to inorganic phosphate supplied across placenta at various fetal weights

The source of the data is given in the text

FETAL WEIGHT	DAILY PER CENT WEIGHT INCREASE	TOTAL P OF FETUS	TOTAL P RETAINED HOURLY IN GROWTH OF FETUS	INORGANIC P SUPPLIED TO FETUS PER HOUR	RATIO P SUPPLIED TO P RETAINED IN GROWTH
gm.		mgm.	mgm.	mgm.	
34	8-10	120	0.4-0.5	0.4	1.0-0.8
63	7-10	260	0.7-1.0	0.9	1.3-0.9
105	7-10	518	1.5-2.2	1.8	1.2-0.8

The quantity of inorganic phosphate reaching the fetus from the maternal plasma is, as is to be seen in table 2, approximately equal to the total phosphate retained by the fetus in its growth. Is the quantity of inorganic phosphate supplied to the fetus sufficient to meet the phosphorus requirements of growth or must a part come from transfer of phospholipid? The data do not permit a precise answer to this question. The guinea pigs used in these experiments came from several independent sources and so were not of a uniform strain. It is consequently not possible to state exactly the fetal growth rate which es-

tablishes the rate at which phosphorus is retained in growth. We have attempted to meet this deficiency by analyzing the data on the basis of growth rates for the fetal guinea pig as derived from *a*, the data of Draper (8), and *b*, the data of Ibsen (9) which are perhaps the most complete in the literature. The range of the daily per cent weight increase as derived from their observations is given in table 2; the higher values in each instance are those of Ibsen. The calculations of table 2 show that on the basis of Draper's observations the inorganic phosphorus found to be transferred across the placenta is sufficient to supply the total phosphorus retained in growth during the period of gestation which is analyzed. Using Ibsen's data, however, there is apparently an appreciable deficiency at all stages which we have studied. We have not presented an analysis from this viewpoint of the three gram fetus since we believe that the deductions are weakened because of the lack of more than a single series of observations made at this stage of growth (table 1 and fig. 4).

DISCUSSION. As has been stated, one of the purposes of this investigation is to attempt to relate the rate of passage of a substance across a unit weight of placenta to the physico-chemical properties of the substance. If the concentration of a normally occurring substance in the maternal plasma be designated as C_{mp} , the total quantity of this substance transferred to the fetus in a unit time as Q_f , and the weight of the placenta as W (W is taken to be proportional to, and therefore representative of, the area of the transfer surface) then:

$$Q_f = kWC_{mp} \quad (2)$$

in which the rate of transfer to the fetus is assumed to be directly proportional to the concentration in the maternal plasma and to the weight of the given placenta. It is evident that the greater the apparent permeability of the placenta to a given substance, the larger will be the value of k .

The coefficient k is made up of many factors. It probably depends upon the permeability of the placenta, i.e., the characteristics of the placenta as a membrane, the presence or absence of secretory activity with respect to the substance by the placenta, and the physico-chemical characteristics of the substance. If it were certain that the placenta performs no secretory work in the transport of material across it and so acted like an inert membrane, k could properly be designated the permeability coefficient. Since the mechanism of transfer is unknown, it appears preferable to designate the coefficient as a transfer coefficient and so to avoid implication as to the intimate nature of transfer.

The transfer coefficients for water, sodium and inorganic phosphate as functions of fetal weight or gestation age are given in figure 5. The value for water ranges from 10 times that of sodium, at a fetal weight of 20 grams, to 16 times, at a fetal weight of 80 grams. The coefficient for inorganic phosphate over the same range of fetal weight is from 2 to 3 times that for sodium.

We have undertaken to compare the physico-chemical properties of ions with their transfer rates across the placenta. Ideally this should be attempted with substances which yield a single species of anion or cation, the physico-chemical properties of which are adequately understood. Such studies are under way

in this laboratory. Phosphate is highly complex. At pH 7.4 and an ionic strength of 0.15, equivalent to that of blood plasma, inorganic phosphate in the concentration found in plasma is about 61 per cent in the form of the ion HPO_4 and 39 per cent in the form of the ion H_2PO_4 . Without a knowledge of whether one or the other or both of these ions traverses the placenta as such, the transfer coefficient k for phosphate as expressed in equation 2 cannot be properly compared to k for sodium without assuming that all the phosphate *anions* cross the placenta in the same ratio as they exist in the plasma. This difficulty does not interfere with the calculation of the rate of transfer of inorganic phosphate as a whole in which it is assumed that the *isotopes* be transferred in the same ratio as in plasma.

In view of the findings on rate of transfer of sodium (2) and water (10) from maternal plasma to fetus as related to the quantities of these substances retained in fetal growth, we have been considerably surprised by the results on inorganic phosphate. In the case of sodium, the fetus receives across the placenta about 50 times as much as is incorporated in the growing tissues and in the case of water about 150 times as much in early stages and 500 times as much in later stages as is retained. Inorganic phosphate, in sharp contrast, is supplied to the fetuses in quantities approximately equivalent to no more than the total phosphorus needed for growth (table 2) and there is no evidence of a factor of safety of any considerable magnitude. The quantity of phosphorus retained per unit time by the fetus is so large compared to the inorganic phosphorus circulating in the maternal plasma that an interesting problem in phosphorus metabolism is raised by the relationship. A 100-gram fetus in each hour of growth retains from 1.5 to 2 mgm. phosphorus (table 2), a quantity about equal to all the inorganic phosphorus in the total plasma of a mother weighing 1200 grams. With a litter of 4, which is not unusual, the phosphorus retained by the fetuses each quarter hour will be equal to the total inorganic phosphorus of the maternal plasma.

These findings and their implications suggest the hypothesis that maternal phosphorus stores are essential in the maintenance of growth during pregnancy and that these stores may be the organic molecules containing phosphorus from which phosphorus is liberated as needed by enzyme activity. In this discussion the phospholipids of the maternal blood plasma have not been considered as an important source of phosphorus for the fetus because of the results of Nielson (11) who found in the rat that the placental transfer of P^{32} when injected in the form of phospholipid is a slow process.

SUMMARY

1. Using radioactive phosphorus as the tracer substance, changes in rate of placental transfer of inorganic phosphate have been measured in guinea pigs from the 31st day of pregnancy until term. The transfer rate of inorganic phosphorus per unit weight of placenta increases about 10 times during this period.

2. Unlike sodium and water which are supplied to the fetus greatly in excess

of the quantities incorporated in growth, inorganic phosphate reaches the fetus from the maternal plasma in an amount only approximately equal to the total phosphorus retained in growth.

3. The placental transfer coefficient for phosphate is 2 to 3 times greater than that for sodium ion, but since salts of phosphate yield two or more anions it appears impossible to compare the coefficient of inorganic phosphate with that of sodium unless it is assumed that the phosphate *anions* cross the placenta in the same ratio as they exist in the plasma.

REFERENCES

- (1) HALPERN, L. J. Biol. Chem. **114**: 747, 1936.
- (2) FLEXNER, L. B. AND H. L. POHL. This Journal **132**: 594, 1941.
- (3) HAHN, L. AND G. HEVESY. Nature **144**: 204, 1939.
- (4) HAHN, L. AND G. HEVESY. Skand. arch. f. physiol. **77**: 148, 1937.
- (5) FLEXNER, L. B., A. GELLHORN AND M. MERRELL. J. Biol. Chem. **144**: 35, 1942.
- (6) HEVESY, G. J. Chem. Soc., p. 1213, 1939.
- (7) FISKE, C. H. AND Y. SUBBAROW. J. Biol. Chem. **66**: 375, 1925.
- (8) DRAPER, R. L. Anat. Rec. **18**: 369, 1920.
- (9) IBSEN, H. L. J. Exper. Zool. **51**: 51, 1928.
- (10) GELLHORN, A. AND L. B. FLEXNER. This Journal **136**: 750, 1942.
- (11) NIELSON, P. E. This Journal **135**: 670, 1942.

DECLINE IN THE RATES OF SWEATING OF MEN WORKING IN SEVERE HEAT

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Prolonged work experiments in severe heat were carried out on men working at a constant rate on a treadmill. Without exception the men's rates of sweating declined during the course of 6-hour experiments in environments severe enough to cause them to sweat at rates of 1200 to 1800 g./hr. in the first hour of the 6-hour period. The real significance of this phenomenon was fully realized when it was found that men failed to maintain thermal equilibrium for 6 hours in some experiments in hot dry environments because their rates of sweating declined, and the amount of sweat secreted was not sufficient to meet their evaporative requirements (Robinson and Gerking, 1946). Pitts, Johnson and Consolazio (1944) and Johnson, Pitts and Consolazio (1944) observed that the rates of sweating of men working in hot environments declined steadily during prolonged exposures. However, they did not describe this phenomenon in detail, and it seems desirable to do so, especially since men's activities in certain hot environments may be limited by a decline in their sweating rates.

PROCEDURE. Six subjects in good physical condition and well acclimatized to the heat performed 6-hour experiments on a treadmill in an air-conditioned room where the air temperature and humidity were kept constant during each exposure. The air movement was 55 m./min. in all experiments. The majority of 50 separate work experiments were carried out with the men walking at 5.6 km. per hour up a 2.5 per cent grade (average MR 190 Cal./m²/hr.), and a few were done at 4.5 km. per hour on the level (average MR 128 Cal./m²/hr.). Several experiments on men sitting at rest (average MR 48 Cal./m²/hr.) for 6 hours were also conducted. The rates of sweating were measured by weighing the men nude before the start of the experiment and at hourly intervals thereafter, urine output and water intake being taken into account. A regular period of 5 minutes was allotted for the weighings at the end of each hour. The subjects maintained water balance during every experiment by drinking measured quantities of 0.1 per cent saline at frequent intervals. The men wore only shorts, shoes, and socks in some experiments while in others they wore poplin tropical uniforms in addition to the previously named garments. These types of clothing were worn in separate experiments by men in both humid and dry environ-

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ments. The data from the work experiments were treated in 4 separate categories; 1, men wearing shorts in dry heat; 2, men wearing poplin in dry heat; 3, men wearing shorts in humid heat, and 4, men wearing poplin in humid heat. Environments in which air temperatures ranged from 31.9 to 38°C. with 95 to 51 per cent relative humidity were considered humid. Dry heat included environments in which the air temperatures ranged from 40.0 to 50.1°C. with relative humidities from 38 to 18 per cent.

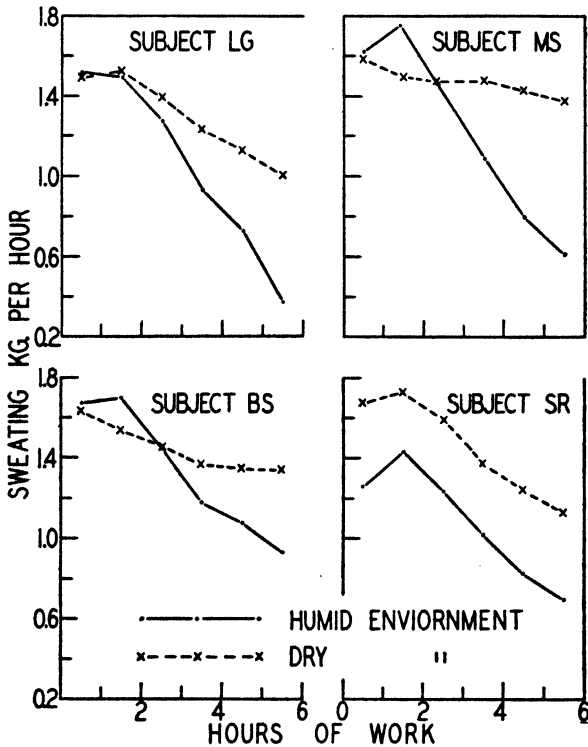


Fig. 1. Decline in the rates of sweating of men working at a MR of 190 Cal./m²/hr. in severely hot environments. The men were fully hydrated in all experiments.

RESULTS. Figure 1 shows the rates of sweating of 4 subjects who performed 6-hour experiments in various severely hot environments. The experiments were all done at a constant rate of work (MR 190 Cal./m²/hr.), and the subjects maintained water balance throughout each experiment. A decline in the rate of sweating of each individual occurred in every experiment shown in the figure, and this decline was much greater in humid than in dry heat. Five of the curves show that the 2nd hour sweating rate was higher than that in the 1st hour, and 3 examples are shown where the reverse is true, i.e., the sweating rate in the 2nd hour is lower than that in the 1st hour. In the entire series of 50 experiments the former result has occurred 29 times and 21 cases of the latter type were observed. For this reason it was felt that the average rate of sweating in the first

2 hours was more truly representative of men's initial response to severe heat than either the 1st or 2nd hour sweating rate. Hereafter, the average rate of sweating in the first 2 hours will be termed the "initial rate of sweating." In all experiments in figure 1 the rate of sweating in the 3rd hour was less than either of the preceding 2 hours. As a general rule this has been the result in the experiments of this kind performed by 6 subjects. In only 3 cases has the sweating rate in the 3rd hour been higher than that in the 1st hour, and in only 2 instances has the 3rd hour rate been higher than that in the 2nd hour. The differences were small (114 g./hr. or less) in both instances. It should be emphasized that the results shown in figure 1 represent the response of fully hydrated subjects and this response is typical of all 6 subjects working at a constant rate in severely humid and dry heat.

As the data were being analyzed it appeared that the greatest declines in the rates of sweating occurred in those experiments in which the men's initial rates of sweating were greatest, i.e., the difference between the initial sweating rate and the sweating rate in the 6th hour became greater as the initial sweating rate increased. The coefficient of correlation between these values was calculated for each of the 4 categories listed above. The data and results of the calculations are presented in table 1. As indicated by the values of r (coefficient of correlation), the initial rate of sweating and the decline show a significant positive correlation for men wearing poplin in both humid and dry heat. There was no significant correlation between these variables for men wearing shorts in either humid or dry heat, but this may have been because the sample was too small. Values of correlation coefficients were considered significant when they had a 95 per cent probability of being real. The results of all 50 of the work experiments at two metabolic rates are shown in table 1. When the 5-hour experiments and those performed at 128 Cal./m²/hr. (starred data in table 1) were eliminated from the calculations, the correlations remained practically the same.

Data in table 2 show that the decline in sweating of individual men was practically identical in separate experiments when their initial rates of sweating, clothing, and the type of environment were the same. Equal initial rates of sweating were used in the comparisons since it was shown above that the decline is related to the initial rate of sweating. It was not possible to represent all the subjects in the 4 categories, i.e., men wearing shorts in humid and dry heat and men wearing poplin in both types of environments; but the decline in sweating rates of those 4 subjects shown in the table is nearly constant for each category. In 5 out of 9 comparisons of table 2 the difference in the decline in sweating rates between experiments was less than 5 per cent and the range of the difference was from 1 to 15 per cent. The experiments were conducted for another purpose, and it is possible that even better agreement might have been found had they been carried out to demonstrate the point under consideration. Two comparisons are included where subject L. G. worked at 2 metabolic rates (190 and 128 Cal./m²/hr.). These comparisons are valid since the decline in sweating rate occurs at both metabolic rates and equal initial sweating rates at

TABLE 1

Results of 50 prolonged work experiments at 2 metabolic rates (190 and 128 Cal./m²/hr.)
 The men were fully hydrated and the environmental conditions remained constant during each experiment

HUMID HEAT—MEN WEARING POPLIN					DRY HEAT—MEN WEARING POPLIN				
Subj.	Dry bulb	Wet bulb	Rate of sweating		Subj.	Dry bulb	Wet bulb	Rate of sweating	
			Initial	Decline*				Initial	Decline*
	°C	°C	g./hr.	g.		°C	°C	g./hr.	g.
L. G.	37.9	28.9	1116	360	L. G.	40.6	27.2	1057	302
M. S.	32.0	31.0	1214	436	L. G.	40.5	27.0	1079	297
L. G.	32.0	31.0	1292	630	L. G.†	44.9	24.1	1201	176
L. G.	32.0	31.0	1366	777	L. G.†	44.9	24.4	1234	364
L. G.†	34.0	32.9	1379	639	M. S.†	44.8	24.5	1342	132
L. G.	31.9	31.1	1409	786	L. G.	45.0	27.2	1377	434
L. G.†	34.5	33.7	1424	756	M. S.†	45.0	24.4	1385	145
L. G.	32.9	32.0	1503	1132	M. S.†	50.1	28.4	1385	243
A. J.	37.9	28.9	1512	506	A. J.	40.6	27.2	1411	266
M. S.	31.9	31.1	1532	637	A. J.	40.5	27.0	1431	334
L. G.	33.0	32.1	1612	867	L. G.	50.0	28.0	1505	502
M. S.	32.9	32.0	1650	698	S. R.†	50.0	31.1	1523	485
M. S.	33.0	32.1	1688	1084	S. R.	50.1	27.4	1606	473
B. S.	37.9	29.4	1689	753	B. S.†	50.0	29.4	1706	664
B. S.	38.0	30.9	1811	751	A. J.	45.0	27.2	1708	470
B. S.	38.0	31.0	1847	930					
$r = 0.613$ (significant)					$r = 0.644$ (significant)				
HUMID HEAT—MEN WEARING SHORTS					DRY HEAT—MEN WEARING SHORTS				
M. S.	37.9	31.5	983	217	L. G.	50.0	27.6	1289	221
L. G.	34.0	32.6	1078	458	L. G.	50.1	28.8	1294	187
W. K.	37.9	31.4	1089	360	L. G.	45.2	31.6	1308	361
M. S.	38.0	31.9	1277	265	L. G.	50.0	30.0	1371	374
L. G.†	35.0	34.3	1317	899	L. G.	49.8	29.3	1388	213
A. J.	37.9	31.5	1328	387	B. S.	50.1	28.1	1439	97
S. R.	34.0	32.6	1330	639	M. S.	50.0	29.0	1486	92
S. R.	34.6	33.9	1344	657	M. S.†	50.0	29.7	1538	163
L. G.	37.9	31.4	1357	583	B. S.	49.9	27.9	1583	239
B. S.	38.0	31.9	1450	503					
$r = 0.547$ (not significant)					$r = 0.392$ (not significant)				

* Initial rate of sweating minus rate of sweating in the 6th hour.

† Experiments were carried out at 128 Cal./m²/hr.

‡ Experiments were of 5 hours' duration. Values of the decline were calculated from extrapolated values of sweating in the 6th hour.

both levels of work can be produced by varying the heat stress imposed on the subjects.

One of the most interesting features of figure 1 is the difference between the sweating rates in humid and dry heat, i.e., when the initial rates of sweating are

TABLE 2

Comparisons of the decline in the rates of sweating of men when their initial rates of sweating, clothing, and type of environment were the same

M. R. 190 Cal./m²/hr. except where specified

SUBJECT	ENVIRONMENT			CLOTHING	RATE OF SWEATING		DECLINE
		Dry bulb	Wet bulb		Initial	6th hr.	
		°C.	°C.		g./hr.	g.	%
L. G.....	Dry	50.1	28.8	Shorts	1294	1107	15
L. G.....	Dry	50.1	27.6	Shorts	1289	1068	17
Difference.....							2
L. G.....	Dry	50.0	30.0	Shorts	1371	977	27
L. G.....	Dry	49.8	29.3	Shorts	1388	1175	15
Difference.....							12
S. R.....	Humid	34.0	32.6	Shorts	1330	691	48
S. R.....	Humid	34.6	33.9	Shorts	1344	687	49
Difference.....							1
A. J.....	Dry	40.6	27.2	Poplin	1411	1145	19
A. J.....	Dry	40.5	27.0	Poplin	1431	1097	25
Difference.....							6
L. G.....	Dry	40.6	27.2	Poplin	1057	755	29
L. G.....	Dry	40.5	27.0	Poplin	1079	782	28
Difference.....							1
M. S.....	Dry	44.8	24.5	Poplin	1342	1210	10
M. S.....	Dry	45.0	24.4	Poplin	1385	1240	11
Difference.....							1
L. G.....	Dry	44.9	24.4	Poplin	1234	870	30
L. G.....	Dry	44.9	24.1	Poplin	1201	1025	15
Difference.....							15
L. G.....	Humid	32.0	31.0	Poplin	1366	589	57
L. G.*.....	Humid	34.0	32.9	Poplin	1379	740	46
Difference.....							11
L. G.....	Humid	31.9	31.1	Poplin	1409	623	56
L. G.*.....	Humid	34.5	33.7	Poplin	1424	668	53
Difference.....							3

* Subject L. G. walked at a M. R. of 128 Cal./m²/hr. in these experiments.

constant, the decline in sweating is much greater in humid than in dry heat. This result might be attributed to variation in the response of the subjects from day to day, but since it was shown above that the decline in sweating of individual men is practically the same in separate experiments carried out under the same conditions, this is not justified. In table 3, experiments were paired which involved the same individual whose initial rate of sweating was the same in a humid environment and in a dry environment, the clothing and work remaining

TABLE 3

Effects of humid and dry environments on the decline in the rates of sweating of men when their initial rates of sweating and clothing were the same. M. R. 190 Cal./m²/hr.

SUBJECT	ENVIRONMENT			CLOTHING	RATE OF SWEATING		DECLINE
		Dry bulb	Wet bulb		Initial	6th hr.	
		°C.	°C.		g./hr.	g.	%
B. S.....	Humid	38.0	31.9	Shorts	1450	947	37
B. S.....	Dry	50.1	28.1	Shorts	1439	1342	7
Difference.....							30
L. G.....	Humid	37.9	31.4	Shorts	1357	774	43
L. G.....	Dry	49.8	29.3	Shorts	1388	1175	15
Difference.....							28
L. G.....	Humid	32.9	32.0	Poplin	1503	371	75
L. G.....	Dry	50.0	28.0	Poplin	1505	1003	33
Difference.....							42
L. G.....	Humid	32.0	31.0	Poplin	1366	589	57
L. G.....	Dry	45.0	27.2	Poplin	1377	943	32
Difference.....							25
L. G.....	Humid	32.0	31.0	Poplin	1292	662	49
L. G.....	Dry	44.9	24.4	Poplin	1234	870	30
Difference.....							19

constant. These paired experiments show a consistent difference in the decline in the rate of sweating between humid and dry heat. The decline was consistently greater by 19 to 42 per cent in humid than in dry heat regardless of whether poplin or shorts were worn. It is unfortunate that the data of only 2 subjects could be used, but we did not have data on the other subjects for this comparison in which their initial rates of sweating were the same. In all experiments regardless of the number of subjects concerned or the initial rate of sweating, the average decline was 21 per cent greater in humid than in dry heat.

The same difference was obtained between the environments when the men wore poplin and when they wore shorts.

An average difference of 13 per cent was found in the decline in the sweating rates between the men wearing shorts and the men wearing poplin. The difference in the decline which was due to the clothing was present in both dry and humid heat.

Thus far most of the experiments discussed have been at a MR of 190 Cal./m²/hr.; however, the data show that the same decline in sweating prevails in men exposed to severe heat at 2 lower metabolic levels. A few experiments at a MR of 128 Cal./m²/hr. can be found in table 1 to illustrate the decline in the rate of sweating which occurs at this grade of work. The rate of sweating of subject M. S. wearing clothes and sitting at rest (MR 48 Cal./m²/hr.) in a humid environment declined from an initial rate of 871 g./hr. to 390 g./hr.

TABLE 4

The rates of sweating of men walking for 6 hours at a M. R. of 190 Cal./m²/hr. in an air temperature of 38.0°C. with 53 per cent relative humidity

The men continued work for a 7th hour with the air temperature at 41.4°C. with 59 per cent relative humidity

SUBJ.	RATE OF SWEATING						
	1st hr.	2nd hr.	3rd hr.	4th hr.	5th hr.	6th hr.	7th hr.
	g.	g.	g.	g.	g.	g.	g.
L. G.....	728	715	704	648	653	662	1091
W. K.....	682	690	697	665	688	697	1341
M. S.....	782	759	750	734	796	767	1432
L. P.....	819	811	773	768	766	767	917
Average.....	753	744	731	704	726	723	1195

in the last hour of a 6-hour experiment. In another sitting experiment the sweating rate of the same subject wearing shorts in a dry environment declined from an initial rate of 946 g./hr. to 841g./hr. in the 6th hour. The decline was observed in every subject who performed 6-hour sitting experiments in extreme heat, but did not occur when the men were exposed to moderate heat. Thus, none of the 6 subjects which we have studied in prolonged experiments in severe heat at 3 metabolic rates have been able to maintain a constant rate of sweating for 6 hours.

The maximal amount of sweat secreted during a period of 6 hours in the experiments reported here is 8771 g. by subject B. S.; the average rate of sweating in this experiment was 1462 g./hr., the highest average which we have recorded. The highest average rates of sweating observed for each of the other 5 subjects during 6-hour experiments are as follows: W. K. 881 g./hr., M. S. 1411 g./hr., L. G. 1296 g./hr., S. R. 1409 g./hr., and A. J. 1442 g./hr. The value on subject W. K. represents the only 6-hour experiment performed on him and is probably not a maximal value. The lowest decline in the rate of sweating in 50

experiments was 6 per cent in a dry environment where the initial sweating rate was 1486 g./hr., and the greatest decline was 75 per cent in a humid environment where the initial sweating rate was 1518 g./hr.

Discussion. When considering the results of the experiments reported above, it must be kept in mind that the conditions were limited to men working at a constant rate in severe heat and do not apply to rates of sweating encountered in temperate or moderately hot environments. For instance, 4 subjects working in an environment in which the dry bulb temperature was 38.0°C . with 33 per cent relative humidity were able to maintain a uniform rate of sweating for 6 hours. The results of these 4 experiments are shown in table 4. Of course, there was individual variation in the response of the men to the environment but each man maintained a fairly uniform rate of sweating for 6 hours in this moderately hot environment. A slight decline in the sweating rates of subjects L. G. and L. P. occurred, but they were able to level off and maintain a constant rate in the later hours of the experiments. Thus, it appears from our data that men can maintain moderate rates of sweating (600 to 800 g./hr.) for long periods of time, but they are not able to sweat at a constant rate of 1200 to 1800 g./hr. for 6 hours in either a severely hot humid or hot dry environment. This means, of course, that there is danger in forcing men to work for prolonged periods of time in extremely hot artificial or natural environments when body temperature measurements are not made periodically. The rates of sweating of men might decline to such an extent that the production of sweat would be insufficient to meet their evaporative requirements, and their body temperatures might rise to a point where heat stroke would be imminent.

Thus far we have been unable to establish the cause for the decline in the rate of sweating of our subjects. It is obvious that some sort of fatigue of the sweating mechanism is involved. To test the possibility of a fatigue factor, a 7-hour experiment was conducted on 4 men in which they worked in a moderately hot environment for 6 hours and the heat stress increased to severe proportions in the 7th hour. The results of this experiment are found in table 4. As previously noted, the men sweated at a constant rate for the first 6 hours, but in the 7th hour when the heat stress was greatly increased, their sweating mechanism responded to the increased demand. It is obvious that fatigue of the sweating mechanism was not great under these conditions. Similar 7-hour experiments in severe heat where the greatest declines in sweating rates occur have not been carried out since the men were usually near exhaustion by the end of the 6th hour.

SUMMARY

The hourly rates of sweating of men walking on a treadmill in severe heat declined steadily during the course of 6-hour experiments. The men were well acclimatized to the heat and maintained water balance by drinking 0.1 per cent saline in the experiments. In 50 experiments the average rate of sweating during the first 2 hours, i.e., initial rate, was 1400 g./hr. and the sweating rates of the men declined from 10 to 80 per cent of this value by the 6th hour, depending

upon environmental conditions. The decline occurred only in relatively high rates of sweating since the men were able to sweat at a practically constant rate (about 750 g./hr.) while working in moderate heat. The declines of the sweating rates were distinctly greater in humid than in dry heat where the initial rates of sweating were about equal. Also, in both humid and dry heat the decline was greater when the men wore Army tropical uniforms than when they wore only broadcloth shorts. Another interesting response shown by men wearing tropical uniforms is that there was a greater decline in their rates of sweating as the initial sweating rates increased. Since the decline in sweating was not associated with dehydration nor with a decreased strength of the stimulus for sweating, it is concluded that the sweating mechanism was fatigued in some way.

REFERENCES

- JOHNSON, R. E., G. C. PITTS AND F. C. CONSOLAZIO. *This Journal* **141**: 575, 1944.
PITTS, G. C., R. E. JOHNSON AND F. C. CONSOLAZIO. *This Journal* **142**: 253, 1944.
ROBINSON, S. AND S. D. GERKING. *Fed. Proc.* **5**: 88, 1946.

THE EFFECT OF NECROSIN ON THE BLOOD SUGAR LEVEL¹

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Several years ago the writer demonstrated in inflammatory exudates the presence of a toxic substance, located in the euglobulin fraction, capable *per se* of reasonably explaining the mechanism of injury accompanying inflammation, i.e., as far as the tissues of the host are concerned (1). Naturally, the irritant in itself may have injurious properties which are thus superimposed upon the action of this toxic substance, in turn liberated by damaged cells. This toxic substance, recovered originally both from canine and from human exudative material (1), has been termed necrosin. Its presence in canine exudates has been confirmed by Smith and Smith who, in addition, have described a closely similar substance in menstrual blood (2). These studies on necrosin have also been confirmed by Tanturi and his collaborators (2a)

Recent studies by the writer have indicated that repeated intravascular injections of necrosin are followed by damage to several visceral organs, notably the liver (3). One of the conspicuous types of damage is the deposition of either fat or glycogen in the liver. In view of the latter finding, it became of interest to determine whether necrosin is capable of influencing carbohydrate metabolism, as revealed by its effect on blood sugar. The present communication indicates that necrosin, as well as whole exudative material, elevates somewhat the blood sugar level. The increase in blood sugar level is not very marked; but nevertheless it is very constant in nature. This finding, as pointed out below, may be of additional significance in our further understanding of the effect of inflammation on the course of diabetes.

EXPERIMENTAL. Necrosin was prepared as described elsewhere (4, 5). Varying doses (table 2) of the material were injected into the circulation of dogs by intracardiac puncture. Subsequently the blood sugar level was followed for several hours, utilizing Folin's method (6). The initial blood sugar concentration was taken prior to any injection of the material. Blood for these determinations was obtained in all cases by nicking small vessels in the ear lobes of dogs.

Prior to the use of necrosin, whole exudative material was injected into the circulation of dogs and the blood sugar level studied for several hours before and after the injection. The results of several such experiments are collected in table 1. It is readily seen that the introduction of 7 to 10 cc. of such material at either alkaline or acid pH is followed by a consistent increase in the blood sugar level. The average increase in six experiments is 23.39 mgm. per 100 cc. (table 1). Blood serum (non-hemolyzed) completely fails to duplicate any such

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effect. The findings support the view that there is something liberated at the site of an acute inflammation *per se* capable of increasing the blood sugar level.

No such effect is obtained with physiological saline or with a concentration of glucose approximately equal to that found in necrosin. Furthermore, when the normal variations in the blood sugar level are studied during approximately five hours, the rise in blood sugar level is negligible, being of the magnitude of 4.25 mgm. per 100 cc.

Finally, various protein fractions of exudative material including dried exudate (which is a means of destroying necrosin) failed to increase significantly the blood sugar level. Necrosin inactivated by boiling or the pseudoglobulin

TABLE 1
Effect of exudate and of blood serum on blood sugar

DOG NO.	pH OF EXUDATE	AMOUNT INJECTED	BASAL BLOOD SUGAR	HIGHEST BLOOD SUGAR 1½ HOURS TO 3 HOURS FOLLOWING INJECTION OF MATERIAL	AMOUNT OF CHANGE IN BLOOD SUGAR
Exudate					
		cc.	mgm./100 cc.	mgm./100 cc.	mgm./100 cc.
94-D	7.6	10	85.11	106.18	+21.07
94-D	7.0	10	84.78	108.40	+23.62
94-D	6.6	10	81.50	109.30	+27.80
94-D	5.9	10	81.80	105.26	+23.46
8-D	7.5	7	85.66	115.94	+30.28
28-D	7.33	7	88.97	103.10	+14.13
Average.....			84.64	108.3	+23.39
Serum					
8-D		7	88.23	91.15	+2.92
28-D		7	95.72	96.15	+0.43
Average.....			91.98	93.65	+1.68

fraction of exudative material was utilized. The latter fraction contains the leukocytosis-promoting factor or LPF of exudates. This substance, as shown in earlier studies, induces a leukocytosis in the circulating blood (7). The albumin fraction of exudates was also employed. None of these protein fractions altered in any appreciable degree the blood sugar level, the average increase being 5.19 mgm. per 100 cc.

On the other hand, when necrosin or the euglobulin fractions of exudates was utilized, an appreciable and consistent increase in blood sugar level occurred (table 2). In twenty experiments the average increase was 19.50 mgm. per 100 cc. This fact would suggest that the rise in blood sugar level induced by the introduction of exudate is referable to the presence of necrosin (cf. table 2), especially since similar results failed to occur with other protein fractions of exudative material.

DISCUSSION. The foregoing findings indicate that whole exudative material is capable, when injected into the circulation of dogs, of increasing the blood

sugar level of such animals. A similar effect can be reproduced only by the active euglobulin fraction of exudate, termed necrosin. These results strongly suggest that necrosin in exudates is responsible for the hyperglycemia-inducing properties of exudative material.³

³ In a previous publication it was pointed out that necrosin also seems to contain some proteolytic activity (10). These studies have been continued with the collaboration of Frederick Bernheim, who conducted a series of studies with the following results:

The experiments were done as follows: Two test tubes were set up, one containing the solution of necrosin, the other the solution of necrosin to which was added fibrinogen (preparation devised at the Harvard Medical School, but made by the Lederle Laboratories) so that 10 mgm. was dissolved in each cubic centimeter. An aliquot was removed from each tube at the beginning of the experiment and the amino nitrogen determined by the Van Slyke method with the use of caprylic alcohol to prevent foaming. A few drops of toluene were added, and both tubes were incubated at 37° for varying periods of time and the amino nitrogen again determined. The initial pH of the necrosin solution varied from 6.5 to 7.7 and did not change significantly during incubation. The following table shows that the incubation of necrosin alone produces only a negligible increase in amino nitrogen and that the addition of fibrinogen produces a greater increase which, since the fibrinogen alone is stable, indicates the presence of some proteolytic enzyme in the necrosin preparation. The proteolysis does not proceed very far because the substances responsible for the extra amino nitrogen are precipitated by 5 per cent trichloroacetic acid.

	INITIAL AMINO-N	FINAL AMINO-N	HOURS OF INCUBATION	REMARKS
	<i>mgm./ cc.</i>	<i>mgm./ cc.</i>		
Necrosin alone.....	0.16	0.17	24	
Necrosin + fibrinogen.....	0.21	0.24		
Necrosin alone.....	0.16	0.16	24	
Necrosin + fibrinogen.....	0.21	0.24		
Necrosin alone.....	0.14	0.16	48	Fairly active necrosin on skin of rabbit
Necrosin + fibrinogen.....	0.23	0.30		
Necrosin alone.....	0.16	0.16	27	Active on skin of rabbit
Necrosin + fibrinogen.....	0.19	0.22		
Necrosin alone.....	0.13	0.12	27	Active on skin of rabbit
Necrosin + fibrinogen.....	0.16	0.16		

In another experiment fibrinogen and necrosin were incubated together at 37° and at 0°. The results are shown in the following table.

HOURS OF INCUBATION	AMINO-N	TEMPERATURE
	<i>mgm./cc.</i>	
0	0.13	37°
4.5	0.14	37°
22.0	0.17	37°
22.0	0.13	0°

Doctor Bernheim's observations essentially substantiate the observations previously described in brief (10), namely, that necrosin seems to possess a slight degree of proteolytic activity.

Earlier studies have indicated that injured cells are capable of producing glucose and that this property is probably of significance in explaining the hyperglycemic tendency which occurs in diabetes when there is a superimposed inflammatory process (8, 9). This view has recently been criticized by Soskin and his associates (11). The technique used by these investigators was somewhat different from that of the author. They compared values in blood serum with those in centrifugalized exudate. Whether the latter procedure does not

TABLE 2
Effect of necrosin on blood sugar level

DOG NO.	DOSE OF NECROSIN	BASAL LEVEL OF BLOOD SUGAR	HIGHEST LEVEL OF BLOOD SUGAR WITHIN 5 HOURS FOLLOWING INJECTION OF NECROSIN	CHANGE IN BLOOD SUGAR LEVEL
	cc.	mgm./100 cc.	mgm./100 cc.	mgm./100 cc.
32-D	10	92.50	109.00	+16.5
32-D	10-12	89.90	132.05	+42.15
32-D	25	102.20	119.75	+17.5
33-D	20	125.44	119.65	-5.77
40-D	6	133.10	154.61	+21.51
40-D	10	94.34	134.68	+40.34
40-D	5	92.40	123.47	+31.07
47-D	5	95.92	125.03	+29.11
42-D	10	103.20	120.12	+16.92
44-D	5	89.68	116.61	+26.93
49-D	5	87.53	129.44	+41.91
22-D	20	101.32	117.52	+16.20
18-D	10 (6 months old inactive necrosin)	96.85	111.43	+14.58
	10 (3 weeks old)	97.08	107.56	+10.48
52-D	10	94.23	114.97	+20.74
	12	97.09	115.27	+18.18
8-D	5	82.27	97.59	+15.32
93-D	12	89.44	105.84	+16.40
93-D	15-19	91.13	108.71	+17.58
22-D	2.5	104.75	86.41	-18.34
	(necrosin and pyrexin)			
Average.....		98.02	117.54	+19.50

favor glycolysis *in vitro* by merely standing during centrifugalization of the exudative material is open to question. The writer compared only some of the constituents of whole blood with those of exudates. These included glucose, lactic acid, and urea. The NPN, the amino acid nitrogen, and the total proteins were compared between whole exudate and serum. This, however, made no difference in the results. In other words, the values with whole blood or with serum showed no essential difference in the mechanism (8). It is therefore somewhat difficult to ascribe any essential difference in results to the difference in cellular content of blood and exudate. Furthermore, it was found that in

diabetic dogs the exudate sugar is higher than that of blood (8). This, on the other hand, is reversed in the case of non-diabetic dogs. Therefore the gradient in the diabetic dogs between exudate and blood could not very well be referred to cellular differences, since that same state of affairs exists in non-diabetic dogs (8, 9).

In view of the above facts, it is still believed that injured cells at the site of an acute inflammation are capable of producing glucose, which in the diabetic animal, owing to the absence of insulin, in turn accumulates and diffuses into the circulation, producing a further state of hyperglycemia. Nevertheless, in view of the present findings that exudative material *per se* and particularly necrosin in exudates is capable of inducing an increase in blood sugar, it is possible that both mechanisms enter into play in the effect of inflammation on the course of diabetes. This would imply that glucose is formed *in situ* at the site of an acute injury, and that necrosin liberated in an inflamed area also favors an increase in blood sugar, presumably by releasing it from the liver.

SUMMARY AND CONCLUSIONS

1. Exudative material injected into the normal circulating blood of a dog induces a rise in its blood sugar level.
2. This effect can be duplicated only by the active euglobulin fraction of exudate, which in turn is or contains a toxic substance known as necrosin.
3. The implications of these findings are discussed in terms of the rôle of inflammation in aggravating diabetes.

REFERENCES

- (1) MENKIN, V. Arch. Path. **36**: 269, 1943.
- (2) SMITH, O. W. AND G. V. SMITH. Proc. Soc. Exper. Biol. and Med. **59**: 116, 1945.
- (2a) TANTURI, C. A., J. F. CANEFA and R. F. BANFI. Revista Medicina **6**: 143, 1945.
- (3) MENKIN, V. Arch. Path. **41**: 376, 1946.
- (4) MENKIN, V. Arch. Path. **39**: 28, 1945.
- (5) MENKIN, V. Science **101**: 422, 1945.
- (6) FOLIN, O. J. Biol. Chem. **77**: 421, 1928.
- (7) MENKIN, V. Arch. Path. **30**: 363, 1940.
- (8) MENKIN, V. This Journal **134**: 517, 1941.
- (9) MENKIN, V. This Journal **138**: 396, 1943.
- (10) MENKIN, V. Am. J. Med. Sc. **208**: 290, 1944.
- (11) MATTAR, E., R. LEVINE AND S. SOSKIN. Federation Proc. **4**: 49, 1945.

APPARENT CURARE EFFECT OF SUBSTANCES THAT DECREASE ACETYLCHOLINE SYNTHESIS¹

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It is currently held that acetylcholine is synthesized in nerve tissue, is released at motor nerve endings, and participates in the production of the effects of indirect stimulation of muscle.

Curare decreases or abolishes the response of striated muscle to indirect stimulation, probably by acting on some structure or process within the muscle. Curare also reduces or abolishes the effect of acetylcholine in inducing muscle shortening.

The action of various substances on the effect of indirect stimulation of striated muscle was tested in the present experiments. (I) A series of compounds, chemically different, but characterized as a group by the fact that they do not decrease the effect of acetylcholine on excised striated muscle (1, 2) and do decrease the synthesis of acetylcholine (3), were used in the first set of experiments; and (II) serum of human blood collected from the working arm was used in the second set of experiments.

EXPERIMENTAL. I. *Action of various compounds on the effect of indirect stimulation of the gastrocnemius muscle.* *Method.* A number of chemical agents were tested as to their effects on *a*, acetylcholine synthesis, and *b*, the response of excised striated muscle to acetylcholine. Of the compounds decreasing the synthesis of acetylcholine without decreasing the response of excised striated muscle to acetylcholine uric acid, pyrrole, alloxan, methylguanidine, vitamin K (menadione), ammonia, hydroquinone, α -naphthol, β -naphthol, and glyceraldehyde were further tested to ascertain their effects on *c*, the response of the gastrocnemius muscle to indirect stimulation.

a. Acetylcholine synthesis. The effects of the compounds on the synthesis of acetylcholine were investigated using a modified method of Quastel, Tennenbaum and Wheatley (4, 5). Mixtures containing varying amounts of the compounds (pH corrected to 7.4), 100 mgm. minced fresh frog brain, 3 mgm. physostigmine salicylate and 3 cc. Ringer's solution were shaken and incubated aerobically for 4 hours at 37°C. After incubation the amount of acetylcholine synthesized was assayed biologically on the sensitized rectus abdominis muscle of the frog. It was also ascertained whether the compounds modified the response of the rectus abdominis muscle to the acetylcholine content of the mixtures during the 2 minutes of immersion for the biological assay by adding the substances to incubated control mixtures after incubation. If the compounds

¹ This study was aided by a grant from the John and Mary R. Markle Foundation.

modified the response of the rectus abdominis muscle to acetylcholine, the changes were taken into account in the calculation.

Calculation. The amount of acetylcholine synthesized was calculated by subtracting from the acetylcholine content of incubated mixtures the acetylcholine content of identical non-incubated mixtures. The amount of acetylcholine synthesized in the control mixtures containing only brain, physostigmine and Ringer's solution was taken as 100 per cent. The acetylcholine content of the mixtures containing the various compounds used was expressed as per cent of the control. All results deviating from 100 per cent by more than twice the square root of the sum of the squares of the standard error of the controls and the standard error of the experiment

$$(2 \sqrt{S.E.^2 (\text{control}) + S.E.^2 (\text{experiments})})$$

were considered significant deviations.

b. *Response of muscle to acetylcholine.* The effects of the same chemical agents on the response of striated muscle to acetylcholine were investigated following a method described previously (1). Shortening of the muscle was induced by immersion in a Ringer's solution containing 50 μg acetylcholine bromide per 100 cc. for two minutes. The shortening of the muscle was registered by an isotonic lever on a kymograph. After stabilization, between two shortenings induced by the acetylcholine solution, instead of washing in Ringer's solution for ten minutes, the muscle was washed only for five minutes and immersed in a series of Ringer's solutions containing one of the chemical agents to be investigated (pH 7), in increasing concentrations, for five minutes.

Control. To ascertain whether changes in the shortening of the muscle observed were due to changes in the sensitivity of the muscle occurring with time, shortening of control muscles, immersed only in Ringer's solution, were induced with the acetylcholine solution as described above. The amount of shortening so induced remained unchanged for at least 3 hours. This period of time was longer than the duration of the experiments described.

Calculation. The amount of shortening of the rectus abdominis muscle after immersion in the solutions of the compounds was expressed as percentage of the amount of shortening of the same muscle before immersion in the solutions of the compounds.

c. *Response of muscle to indirect stimulation.* The action of the substances on the effect of indirect stimulation on striated muscle was investigated as follows. Frogs of 18 to 23 grams' body weight were pithed and pinned to a board. A knee was fixed and the tendon of the gastrocnemius muscle attached to an isotonic lever. Movements of the gastrocnemius muscle were registered by an isotonic lever on a kymograph. Silver electrodes were placed on the intra-abdominal branches of the sciatic nerve. The heart was also exposed. During preparation sections of blood vessels were carefully avoided. The preparation was placed in a humid chamber to avoid drying of the nerve. The sciatic nerve was stimulated for 3 seconds every 30 seconds by means of a thyatron stimulator constructed by Weiss (6). A current of 70 per second frequency and supra-threshold intensity was used for stimulation. After the shortening of the gas-

trocnemius muscle remained constant for at least three successive stimulations 0.2 cc. of the solution of one of the compounds was injected into the ventricle of the heart. The compounds were dissolved in Ringer's solution and the pH corrected to 7. The intermittent stimulation of the sciatic nerve of the frog was continued for 10 to 15 minutes as described above. Upon conclusion of the experiments the frogs were injected with Berlin blue to ascertain whether or not the blood supply of the gastrocnemius muscle remained adequate during the experiments.

Control. Frogs prepared in exactly the same way except that they were injected with 0.2 cc. Ringer's solution served as controls. The shortening of the gastrocnemius muscle remained nearly constant for at least 30 minutes, a period of time longer than that required by the experiments described above. This control was chosen because the effect of most of the compounds used could not be reversed within a reasonably short period of time.

Calculation. The shortening of the gastrocnemius muscle before injection served as 100 per cent, the shortening after injection was expressed as per cent of this value.

RESULTS. *a.* The effect of the compounds on the synthesis of acetylcholine is given in table 1. Except for Ringer's solution, uracil and l(-)leucine, the compounds decreased the acetylcholine synthesis. The decrease varied from 23 to 79 per cent for the greatest concentrations used.

b. The effect of the compounds on the response of the excised rectus abdominis muscle to acetylcholine is given in table 2. Except for d-tubocurarine, the compounds did not decrease the response of the muscle to acetylcholine.

c. The effect of the compounds on the response of the gastrocnemius muscle to indirect stimulation is given in table 3. Uric acid, pyrrole, alloxan, methylguanidine, vitamin K, ammonia, hydroquinone, α -naphthol, β -naphthol and glyceraldehyde decreased the response of the gastrocnemius muscle to indirect stimulation from 27 to 54 per cent.

Two-tenths cubic centimeter of a solution containing 10 mgm. d-tubocurarine per 100 cc. decreased the effect of indirect stimulation during the 10 minutes' test period. On continuation of stimulation for a longer period of time the response of the muscle to indirect stimulation was completely abolished. Injection of 0.2 cc. of a solution containing 100 mgm. d-tubocurarine per 100 cc. abolished the response of the gastrocnemius muscle to indirect stimulation in a few minutes.

To ascertain whether or not the decrease of the response of the gastrocnemius muscle to indirect stimulation observed after injection of the compounds used was due to technical errors, e.g., drying out or deterioration of the preparation, the following experiments were performed. Frogs were injected either with 0.2 cc. Ringer's solution or with 0.2 cc. solution of compounds that do not decrease either the acetylcholine synthesis (table 1) or the response of the excised muscle to acetylcholine (table 2). The results are given in table 3. These compounds did not decrease the response of the gastrocnemius muscle to indirect stimulation indicating that the decrease of the response observed in the

TABLE 1
Effect of the compounds on acetylcholine synthesis

COMPOUNDS	AMOUNT OF ACETYLCHOLINE SYNTHESIZED PER CENT OF CONTROL*			
	Amount of the compounds (mgm.) added to 100 mgm. frog brain			
	3	0.3	0.03	0.003
Uric acid.....	55 \pm 2.0	66 \pm 2.5	88 \pm 2.9	
Pyrrole.....	77 \pm 2.2	83 \pm 2.8	94 \pm 2.1	110 \pm 3.0
Alloxan.....	40 \pm 1.5	59 \pm 2.0	80 \pm 2.3	88 \pm 2.0
Methylguanidine.....	58 \pm 2.3	103 \pm 3.0	100 \pm 2.9	118 \pm 3.1
Vitamin K.....		66 \pm 1.7	74 \pm 2.8	83 \pm 2.7
Ammonia.....	34 \pm 1.7	67 \pm 1.8	90 \pm 2.1	101 \pm 2.0
Hydroquinone.....	40 \pm 2.1	71 \pm 2.8	98 \pm 2.4	98 \pm 3.3
α -Naphthol.....	21 \pm 2.0	50 \pm 2.0	81 \pm 2.0	93 \pm 3.0
β -Naphthol.....	22 \pm 2.5	50 \pm 1.9	84 \pm 2.5	100 \pm 2.8
Glyceraldehyde.....	70 \pm 2.4	90 \pm 3.0	102 \pm 2.2	100 \pm 2.2
d-Tubocurarine.....		45 \pm 1.8	104 \pm 2.3	104 \pm 2.3
Ringer's solution.....	100 \pm 1.2	100 \pm 1.0	100 \pm 0.5	100 \pm 1.0
Uracil.....	105 \pm 2.5	100 \pm 2.0	107 \pm 2.1	100 \pm 2.0
l (-)Leucine.....	106 \pm 2.7	104 \pm 3.2	104 \pm 2.6	103 \pm 3.1

* Each value represents the average of 10 separate experiments.

TABLE 2
Effect of the compounds on the response of the rectus abdominis muscle to acetylcholine

COMPOUND	MAGNITUDE OF SHORTENING IN PER CENT OF CONTROL*					
	Concentrations of the compounds (mgm.) per 100 cc. Ringer's solution:					
	100	10	1	0.1	0.01	0.001
Uric acid.....	105 \pm 2.5	102 \pm 2.0	99 \pm 2.7	105 \pm 1.9	105 \pm 2.0	103 \pm 1.0
Pyrrole.....		142 \pm 3.0	93 \pm 2.2	96 \pm 2.0	99 \pm 1.7	101 \pm 0.7
Alloxan.....		92 \pm 2.5	93 \pm 2.2	98 \pm 1.2	104 \pm 1.8	100 \pm 0.2
Methylguanidine.....		118 \pm 2.0	105 \pm 2.0	103 \pm 1.5	101 \pm 1.1	102 \pm 0.5
Vitamin K.....	162 \pm 4.0	102 \pm 2.2	104 \pm 1.8	100 \pm 1.1	105 \pm 0.8	107 \pm 0.3
Ammonia.....	119 \pm 2.0	96 \pm 1.3	97 \pm 1.9	98 \pm 1.8	105 \pm 0.9	102 \pm 0.5
Hydroquinone.....	s†	185 \pm 3.1	105 \pm 1.5	102 \pm 1.2	99 \pm 1.0	101 \pm 0.4
α -Naphthol.....	s	208 \pm 3.5	124 \pm 1.9	105 \pm 1.5	102 \pm 0.9	100 \pm 1.0
β -Naphthol.....	s	191 \pm 3.2	127 \pm 1.8	114 \pm 1.2	105 \pm 1.1	103 \pm 0.3
Glyceraldehyde.....		100 \pm 2.5	101 \pm 1.7	99 \pm 1.0	96 \pm 0.8	99 \pm 0.9
d-Tubercurarine.....			8 \pm 3.1	49 \pm 2.5	82 \pm 1.9	99 \pm 1.4
Ringer's solution.....	100 \pm 0.6	100 \pm 0.5	100 \pm 0.8	100 \pm 1.5	100 \pm 1.3	100 \pm 0.9
Uracil.....	138 \pm 4.0	105 \pm 2.7	103 \pm 3.0	98 \pm 1.7	97 \pm 1.1	100 \pm 1.0
l (-)Leucine.....	130 \pm 4.2	100 \pm 1.0	99 \pm 1.5	101 \pm 1.0	98 \pm 1.6	100 \pm 1.2

* Each value represents the average of 10 separate experiments.

† "s" means that muscle shortening occurred during the immersion in the solution of the compound for 5 minutes without addition of acetylcholine.

TABLE 3
Effect of the substances on the response of the gastrocnemius muscle of frog to indirect stimulation

SUBSTANCE	CONCENTRATION OF SUBSTANCE IN MGM. PER 100 CC. RINGER'S SOLUTION	NO. OF EXPTS.	MAGNITUDE OF SHORTENING OF THE GASTROCNEMIUS MUSCLE IN PER CENT OF CONTROL									
			Time of stimulation after injection of 0.2 cc. of the solution of the substances in the ventricle of the frog heart (in minutes):									
			1	2	3	4	5	6	7	8	9	10
Uric acid.....	100	10	93 ± 1.9	88 ± 2.4	83 ± 1.5	78 ± 2.0	75 ± 2.0	73 ± 1.8	68 ± 2.7	63 ± 2.0	63 ± 2.6	59 ± 2.3
Pyrrole.....	50	10	95 ± 0.9	90 ± 1.5	85 ± 1.9	80 ± 1.1	77 ± 1.5	73 ± 1.9	70 ± 2.0	65 ± 1.7	62 ± 2.8	60 ± 2.6
Alloxan.....	100	10	90 ± 1.8	85 ± 2.0	81 ± 2.0	75 ± 2.8	75 ± 3.0	71 ± 2.7	68 ± 2.2	66 ± 2.7	63 ± 3.0	57 ± 3.6
Methylguanidine.....	100	15	95 ± 1.4	87 ± 1.2	82 ± 1.4	80 ± 1.7	76 ± 2.0	75 ± 2.0	67 ± 2.5	60 ± 2.0	58 ± 2.4	53 ± 2.2
Vitamin K.....	100	15	93 ± 2.0	90 ± 2.4	85 ± 2.2	81 ± 2.3	72 ± 2.6	63 ± 2.7	56 ± 2.7	54 ± 2.9	50 ± 3.2	48 ± 4.0
Ammonia.....	100	10	92 ± 1.5	89 ± 1.9	84 ± 2.2	80 ± 2.2	75 ± 1.8	64 ± 3.0	61 ± 3.0	58 ± 3.7	55 ± 4.0	52 ± 4.2
Hydroquinone.....	100	12	95 ± 1.8	87 ± 1.2	76 ± 1.3	69 ± 2.3	66 ± 2.7	63 ± 3.1	63 ± 2.9	60 ± 2.8	52 ± 3.0	50 ± 4.0
α-Naphthol.....	100	10	92 ± 1.0	88 ± 1.3	83 ± 1.5	74 ± 1.9	69 ± 2.5	62 ± 2.9	58 ± 2.2	54 ± 1.8	52 ± 2.0	48 ± 2.3
β-Naphthol.....	100	10	94 ± 1.1	90 ± 2.0	86 ± 2.2	76 ± 2.2	68 ± 2.5	60 ± 2.0	56 ± 3.3	53 ± 2.8	50 ± 2.9	46 ± 2.7
Glyceraldehyde.....	100	10	95 ± 0.9	92 ± 1.0	90 ± 1.2	90 ± 1.7	86 ± 1.9	83 ± 1.3	82 ± 2.0	80 ± 1.2	76 ± 1.5	73 ± 1.9
d-Tubocurarine.....	10	12	97 ± 1.1	92 ± 1.2	86 ± 1.1	82 ± 1.2	75 ± 1.8	75 ± 1.8	71 ± 1.3	64 ± 1.9	56 ± 2.5	48 ± 2.6
Ringer's solution.....		60	99 ± 0.3	98 ± 0.5	100 ± 0.4	100 ± 0.7	102 ± 0.2	102 ± 0.2	98 ± 0.5	100 ± 0.7	102 ± 0.6	100 ± 0.5
Uracil.....	100	10	105 ± 0.8	101 ± 0.8	100 ± 1.3	99 ± 0.6	97 ± 1.7	97 ± 0.5	94 ± 0.6	96 ± 0.9	95 ± 1.4	94 ± 1.2
l(-)Leucine.....	100	10	100 ± 0.9	98 ± 1.0	97 ± 0.9	99 ± 0.8	100 ± 1.0	99 ± 1.2	99 ± 1.1	97 ± 0.8	98 ± 1.3	95 ± 1.1
Serum (immobilized arm).....		12	100 ± 0.7	99 ± 0.9	99 ± 0.9	98 ± 0.6	98 ± 1.4	99 ± 0.4	99 ± 0.6	99 ± 0.9	100 ± 0.4	97 ± 0.4
Serum (working arm).....		12	97 ± 0.2	94 ± 0.3	92 ± 0.7	90 ± 0.5	88 ± 0.7	87 ± 1.0	83 ± 1.2	82 ± 1.3	80 ± 1.1	78 ± 1.2

presence of the first group of chemical agents was due to some influence of those compounds on the preparation.

II. *Action of serum on the effect of indirect stimulation of the gastrocnemius muscle.* The compounds dealt with in the first set of experiments either do not exist in the body, or, if metabolites, they are usually not present in high enough concentrations to cause a significant decrease of the response of muscle to indirect stimulation. Since serum collected from the fatigued arm of healthy subjects has been shown to decrease the synthesis of acetylcholine (7), it seemed desirable to ascertain its effect on the response of muscle to indirect stimulation.

Method. Experiments were performed on 12 healthy subjects as previously described (8). The subjects alternately flexed their fingers, forming a fist, and extended them fully, performing the entire action rhythmically, at the rate of one contraction per second. A sphygmomanometer cuff was applied to both the working and the immobilized arm and inflated during the fourth to ninth contraction of the fingers to a pressure of 250 mm. Hg. The flexion and extension of the fingers was continued until severe fatigue occurred. Afterwards 10 cc. of blood was collected from the brachial vein. The blood collected from the immobilized arm served as the control specimen. The blood was centrifuged immediately after collection and the effects of serum on *a*, acetylcholine synthesis; *b*, response of excised muscle to acetylcholine, and *c*, response of muscle to indirect stimulation were investigated as described previously.

a. The effect of serum on the synthesis of acetylcholine was ascertained by adding 1 cc. of serum to the mixture of brain, physostigmine salicylate, and Ringer's solution before incubation.

b. The effect of serum on the response of the excised rectus abdominis muscle to acetylcholine was investigated by immersion of the muscle for five minutes in serum before immersion in the solution of acetylcholine.

c. The effect of serum on the response of the gastrocnemius muscle to indirect stimulation was ascertained by the method described previously (injecting 0.2 cc. of serum into the ventricle of the frog heart.).

RESULTS. *a.* The amount of acetylcholine synthesized in the presence of 1 cc. of serum collected from the working arm was 40 ± 1.6 per cent less than in the presence of 1 cc. of serum collected from the immobilized arm (average of 12 expts.).

b. The response of the excised rectus abdominis muscle to acetylcholine was similar for both muscles immersed in serum from the immobilized arm and muscles immersed in serum from the working arm (average of 12 expts.).

c. Serum collected from the immobilized arm did not decrease the response of the gastrocnemius muscle to indirect stimulation, while serum collected from the working arm decreased the response of the muscle to indirect stimulation by 22 ± 1.2 per cent (average of 12 expts.) (table 3).

DISCUSSION. It has been shown that there are substances that, in contrast to curare, decrease the response of striated muscle to indirect stimulation without decreasing the response of the excised muscle to acetylcholine. These chemically very different substances were tested for their effect on the response of muscle to indirect stimulation because they were found to decrease the syn-

thesis of acetylcholine (3) without decreasing the response of the excised muscle to acetylcholine (1, 2). The choice of such chemically different substances minimizes the chance that the decrease of response to indirect stimulation was induced by some mechanism other than by decrease of acetylcholine synthesis.

These data may illuminate observations made on patients with myasthenia gravis and may modify their interpretations. In 1934 Nevin suggested (9) that a metabolite with a curare-like action is responsible for the symptomatology observed in patients with myasthenia gravis. This curare-like agent would prevent the response of the receptor mechanism within the striated muscle to acetylcholine. In 1943 it was demonstrated that serum of blood and spinal fluid (5, 10) from patients with myasthenia gravis contains agents that decrease acetylcholine synthesis, and that the decrease of acetylcholine synthesis is proportional to the gravity of the symptomatology. It was also shown that the response of muscle to acetylcholine is not modified by the presence of serum collected from patients with myasthenia gravis (11). The results presented in this communication suggest that the agent responsible for the decrease of acetylcholine synthesis may be responsible for the apparent curare effect observed.

SUMMARY

1. The effect of a group of substances on the response of the gastrocnemius muscle of the frog to indirect stimulation was investigated. These substances were found to decrease the synthesis of acetylcholine, but they did not decrease the response of excised striated muscle to acetylcholine. In this their action differed from that of curare.

2. Uric acid, pyrrole, alloxan, methylguanidine, vitamin K, ammonia, hydroquinone, α -naphthol, β -naphthol, and glyceraldehyde decreased the response of striated muscle to indirect stimulation.

3. Serum collected from the working arm of humans also decreased the response of striated muscle to indirect stimulation and decreased the synthesis of acetylcholine without decreasing the response of excised muscle to acetylcholine.

4. It is suggested that an apparent curare effect may be exerted by substances acting not on some structure or process within the muscle, but on the nerve tissue.

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REFERENCES

- (1) TORDA, C. AND H. G. WOLFF. This Journal **145**: 419, 608, 1946.
- (2) TORDA, C. AND H. G. WOLFF. J. Exper. Med. and Surg. **4**: 50, 1946.
- (3) TORDA, C. AND H. G. WOLFF. Science **103**: 645, 1946.
- (4) QUASTEL, J. H., M. TENNENBAUM AND A. H. WHEATLEY. Bioch. J. **30**: 1668, 1936.
- (5) TORDA, C. AND H. G. WOLFF. J. Clin. Investigation **23**: 649, 1944.
- (6) WEISS, W. I. Electronics **19**: 2, 1946.
- (7) TORDA, C. AND H. G. WOLFF. Proc. Soc. Exper. Biol. and Med. **59**: 13, 1945.
- (8) TORDA, C. AND H. G. WOLFF. Proc. Soc. Exper. Biol. and Med. **58**: 242, 1945.
- (9) NEVIN, S. Brain **57**: 239, 1934; J. Neurol. and Psychiatry, **1**: 120, 1938.
- (10) TORDA, C. AND H. G. WOLFF. Science **98**: 224, 1943; **100**: 200, 1944.
- (11) COHEN, S. J., J. C. KAUFMAN, E. B. SPITZ AND M. M. STERN. Proc. Soc. Exper. Biol. and Med. **58**: 181, 1945.

SWEAT GLAND ACTIVITY AND CHANGING PATTERNS OF SWEAT SECRETION ON THE SKIN SURFACE

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As pointed out by Richter and Whelan (1943) present information on sweat gland activity is limited, particularly because of inadequate methods of measuring sweat responses. Secretory activity of individual glands and groups of glands in a small circumscribed area (other than on the finger pad) has not been described clearly. Information upon this function would supplement present knowledge on the nature of the nervous control of sweating.

METHOD. A method was recently described whereby the secretion of individual glands can be studied (Randall, 1946). Application of dilute iodine solution (3 per cent iodine in alcohol) to the test area gives a characteristic starch-iodine reaction when ordinary starch containing paper is pressed lightly over the area. The activity of individual glands is designated by a blue-black spot on the test paper at each point of contact between paper and secreting sweat pore. The size of the spot is a qualitative measure of the amount of sweat secretion.

In methods hitherto described most of the investigations of individual gland activity have been carried out upon the sweat glands of the palm and finger pads. It is known, however, that thermal sweating in these areas is complicated by psychic influences, and possibly by vasomotor activity. The precise relationship between vasomotor and sudomotor control in the periphery is not understood, therefore in order to limit this study to sudomotor influences as much as possible, we chose to carry out these early experiments upon the extensor surface of the forearm where variations in vasomotor activity are relatively restricted. This area affords an additional advantage in that the sweat glands are less numerous than on the palmar surfaces of the hand, thereby making counts easier and more accurate. Successive twenty second records were taken at the beginning of each minute or half minute.

RESULTS. *Spontaneous cycles of sweat gland activity:* It is apparent from figure 1 that at room temperatures between 20 to 27°C the sweat glands are not continuously emptying onto the surface but tend to show periods of relatively great activity alternating with periods of little or no activity. These periodic phases of sweating show considerable variation in different individuals as well as in the same individual at different times and at different temperatures. At the lower environmental temperatures in figure 1 the peaks of sweating activity are sharp and separated by many minutes of complete absence of sweating. That is, there are periods in which a number of glands are suddenly activated, they empty onto the skin surface for a few seconds and then cease their secretory activity. The duration of activity of the individual glands is revealed in experiments in which the continuous activity of glands in a small area is recorded by leaving

the test papers in place for 15 seconds, allowing 5 seconds to elapse after this exposure, and then taking succeeding records at similar intervals until several sweating cycles are completed. At environmental temperatures between 20 to 30°C no sweat gland on the forearm has been observed to secrete continuously for a period longer than 45 seconds. Usually at these temperatures, activity of the individual gland is limited to 15 seconds or less. The method as it is now used does not permit interpretation of activity of the glands during the fifteen or twenty seconds' exposure. That is, it is not as yet known whether one droplet appears and is absorbed on the paper or whether several droplets appear during a 15 second period, the final sweat spot representing the additive effect of several

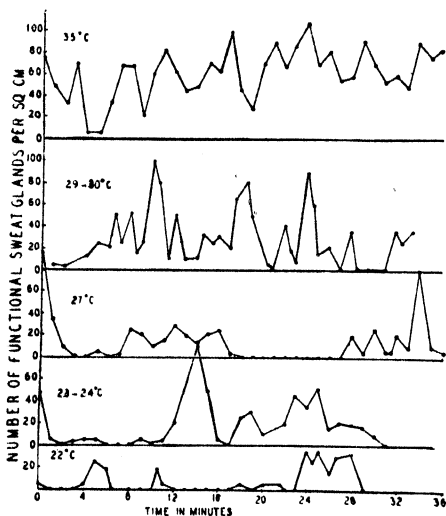


Fig. 1

Fig. 1. Periodic activity of the sweat glands on the extensor surface of the forearm of a subject at different environmental temperatures.

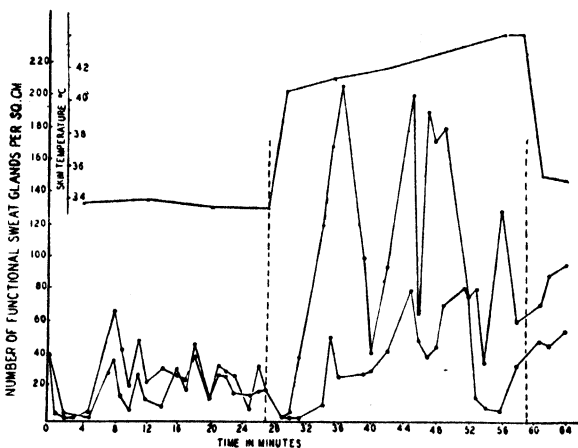


Fig. 4

Fig. 4. Bilateral sweat response with application of radiant heat to one arm. Open circles represent sweat glands on heated arm; closed circles represent sweat glands on control (unheated) arm. Heat applied during interval indicated between broken lines. Skin temperature recorded by unshielded thermocouple placed on heat area.

droplets. In either event, such studies indicate that only a small proportion of the sweat glands empty their secretion onto the surface and are then functionally replaced by other glands in the immediate vicinity, or sweating in this area may cease entirely. Some glands may secrete only once while others may secrete several times during one sweating cycle. It also appears that certain glands function repeatedly during successive cycles while others function much less frequently.

As the environmental temperature is elevated the interval between periods of activity decreases and the basal number of active glands tends to rise until there is only an occasional fall to zero. Simultaneously it may be observed that the

peaks often become higher and longer sustained. Under conditions of relatively extreme thermal stimulation over a large area (as in a hot tub bath, 40–44°C.) some cyclic activity continues but is superimposed upon a basal activity which includes so many glands that the alternations between high and low points are relatively shallow. Peaks of activity in such experiments approach the maximum number of functional sweat glands in a given area. Under conditions of profuse sweating (maximum number of sweat glands plus large output per gland) coalescence of sweat spots make accurate counts more difficult and some of the

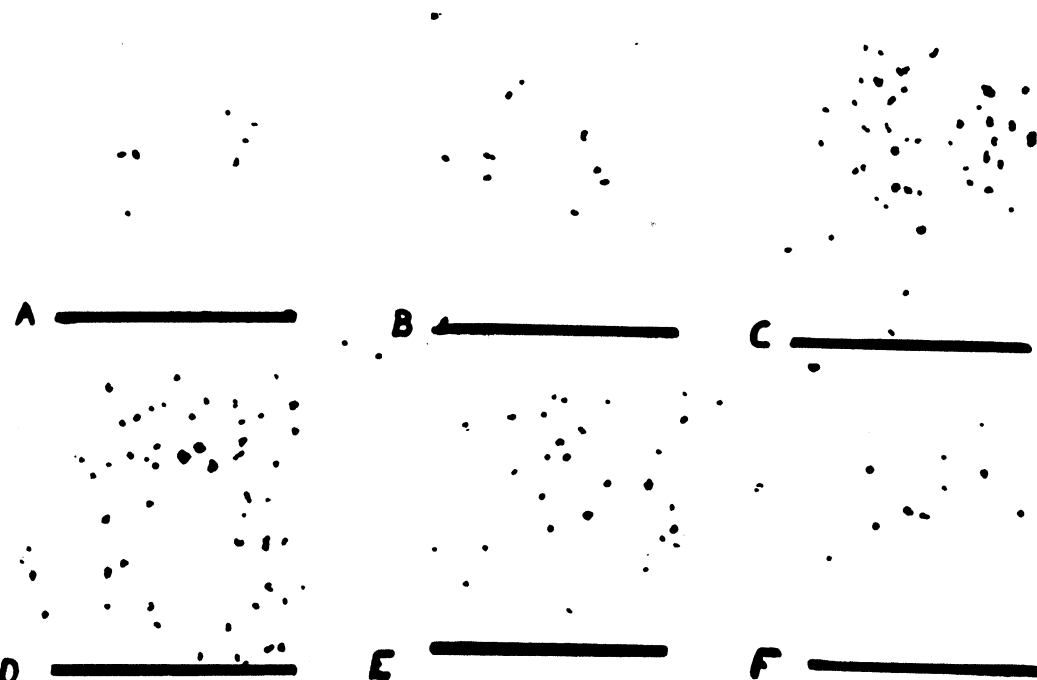


Fig. 2. Enlarged photograph of sweat spots showing development and regression of a cycle of sweat gland activity on the forearm during strong visceral sensation. Calibration line represents one centimeter. For purposes of reproduction it was necessary to retouch with india ink a photograph of the original record.

cyclic activity may be obscured. Careful observation under these conditions, however, gives evidence that the phasic alternations continue.

The periodicity of active and inactive phases has not proved to be uniform but is influenced by such factors as environmental (and probably blood) temperature, strong peripheral stimulation, and psychic or emotional excitement. It is well known that the effects of the latter type of stimulation are particularly prominent on the palmar and plantar surfaces. Strong visceral sensations may also result in excitation of peaks of sweating. Such stimulation is illustrated in figure 2 in which experiment the subject experienced a strong desire for defecation during the course of the experiment. Here there was a progressive development of a

high peak of sweating during the period of sensation (segments A, B, C and D) and a gradual decrease in number of active glands as the sensation regressed (segments E and F). This experiment is of additional interest because of the time required for the development and regression of the complete cycle. The succeeding records were taken at one minute intervals with two minutes between E and F. In other words, seven minutes were required for the completion of the cycle as compared with a few seconds or one to two minutes required for most cycles in the resting, undisturbed subject. Similarly prolonged cycles are sometimes observed when the patient receives certain psychic or emotional stimulation. In fact, when such prolonged phases are observed, some intrinsic or extrinsic stimulation may be suspected. Such a cycle is apparent in the 23–24°C. range of figure 1.

In carrying out these experiments the attention of the subject was often diverted from the experiment, and he was not objectively aware of the peaks of sweating. The experimenter was often surprised, therefore, by accurate predictions such as “now I’m sweating” or “there, I feel sweaty” occasionally volunteered by the subject. The sensation of sweating occurred simultaneously with the appearance of increased numbers of functional sweat glands on several body surfaces, but was usually localized by the subject to the face and forehead. The sensation is not unlike that which is often experienced when sweating suddenly appears upon the face and forehead after eating hot or spiced foods (gustatory sweating). It is not a sensation of cooling such as might be expected if it were caused by evaporation. The “feeling” persists throughout the peak of sweating and disappears with the regression of the cycle.

Graded responses to sweat gland stimulation: In experiments designed to study conditions of profuse sweating it has been observed that once the maximum number of sweat glands is reached, further increases in the total amount of sweat may be attained by increased output by the individual glands. This is demonstrated by a progressive increase in sweat output as the body is warmed in a hot bath. The small pin-point size of the sweat spots before and immediately after exposure to hot water is sharply contrasted with the large diffuse spots which become evident as the exposure continues (fig. 3). In this experiment variation from 0 to 170 active sweat pores per square centimeter characterized the cyclic phases of sweating during a seventeen minute control period. At 17½ minutes in the protocol the subject was seated in a water bath at 42°C with the body immersed to the level of the umbilicus. The stimulus thus presented was severe and applied to a large area. Thirty seconds after immersion only 30 sweat pores were filled, and at the beginning of the 19th minute (segment A, fig. 3) 150 pores were filled. This number was not in excess of other peak values recorded during the control period, however, and was followed by a count of 85 secreting pores at the 20th minute (segment B). Following this record, 238 or nearly the maximum number of sweat glands for this area were active (segment C), and allowing for small phasic oscillations which persisted, this number of glands remained functional throughout the remainder of the exposure. During this period the output of the individual glands (as indicated by the size of the spots) increased slightly

through segment D (22 min.). Markedly increased output is evident in segment E (24 min.), and still further increased in segment F (28 min.).

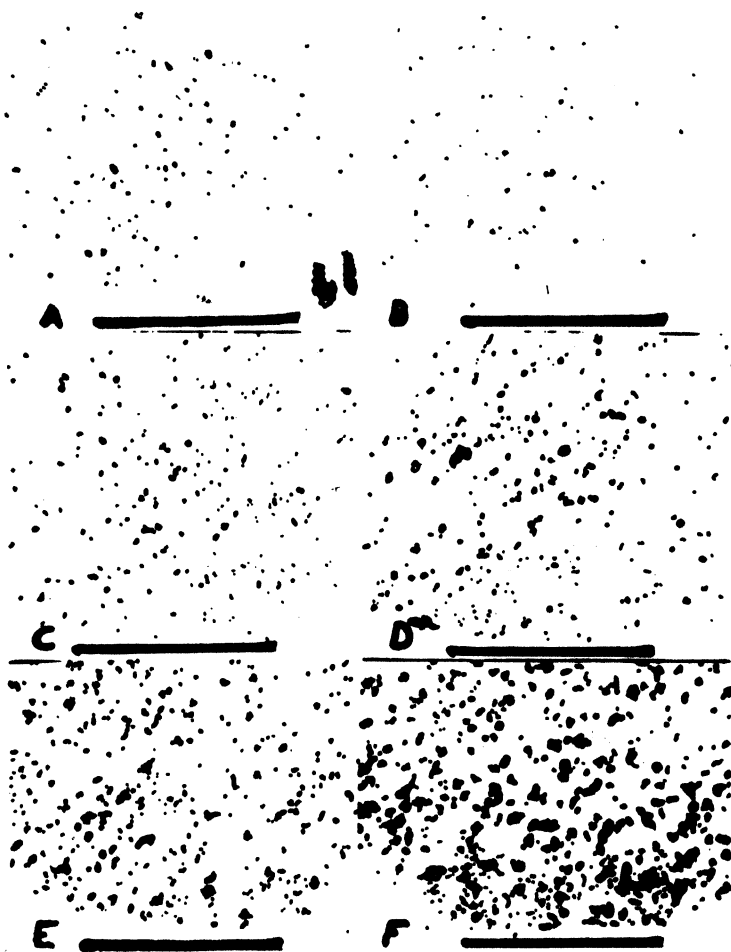


Fig. 3. Enlarged photograph showing sweat spots on the forearm while the subject is seated in a hot water bath (42°C). All segments of equal exposure; calibration line represents one centimeter. Note first progressive increase in number of functional glands followed by increase in size of spots (increased output per gland). Time relationships outlined in text. For purposes of reproduction it was necessary to retouch with india ink a photograph of the original record.

It might be argued here that the size of a sweat spot appearing on the test paper may not be attributed entirely to the quantitative output of the individual sweat pore in a unit of time, since it is probable that many pores are activated during the latter part of the exposure and thus should not be considered to have been exposed throughout the entire period. Such an argument undoubtedly holds in records of spontaneous sweating in the resting, undisturbed subject. Experience with profuse sweating responses however (ther-

mal, mecholyl, and nerve stimulation experiments) has given evidence that such graded levels of individual gland output (as illustrated in fig. 3) is a real phenomenon.

This experiment emphasizes several aspects of the physiological activity of the sweat glands. There is a definite time lag ($3\frac{1}{2}$ min. in this experiment) between application of the stimulus and the activation of large numbers of glands in the test area. During this time lag, phasic oscillations in the number of active glands continue. In our experience this lag has varied between 1 and 5 minutes, being somewhat shorter with more severe stimulation. Such a lag certainly excludes the possibility of a rapid reflex response to peripheral stimulation as it is ordinarily understood. Following this lag, large numbers of glands are brought into activity but it is not until several minutes later that significant increase in sweat output by individual glands occurs.

Adolph recently described the time course of the rate of evaporative loss of sweat (1946) which would appear to correspond to the two stages in sweating responses reported here. An initial sweating in which evaporative loss is increased by no more than $2\frac{1}{2}$ -fold in Adolph's experiments seemingly corresponds to the initial increase in number of participating glands in our experiments. A later, 10-fold increase in sweating (Adolph) would then appear to correspond to the delayed increase in output of each active gland in our experiments. In the experiment described (fig. 3) a rise of 1°F in oral temperature occurred during the latter part of the exposure, and it seemed likely that a rise in blood temperature bathing the sweat centers could be the functional cause of the second, profuse stage of sweating.

The occurrence of large diffuse sweat spots is limited almost exclusively to severe thermal stimulation, and such spots compare in size to the large spots observed when the glands are directly activated by acetyl-beta-methylcholine. This drug not only stimulates a maximum number of glands when administered into the dermal layers by iontophoresis, but it also produces very large spots. These spots are many times as large as those occurring during the normal phasic secretory cycles in a comfortable environment. The experiments therefore suggest a mechanism available to the body for fine and gross regulation of heat loss by evaporation under a variety of environmental temperatures and conditions.

Locally and reflexly induced sweating: Simultaneous records from similar areas of the right and left forearms demonstrate that phasic variation in number of functional sweat glands is bilateral. Although the correlation does not appear to be exact (fig. 4) close correspondence in phasic activity is evident in both arms during the control period as well as during the application of radiant heat to one arm. A bilateral discharge from sweat centers in the central nervous system is therefore indicated. These phasic variations persist even though the basal number of active glands increased and even though the heating was continuous. Again, a decided lag in excitation of sweating is observed following the application of the stimulus, and it is also evident that the lag is more pronounced on the control (unheated) arm. Skin temperature recorded by thermocouple placed on the heated area increased rapidly and the subject immediately experienced a

sensation of heat which in some experiments became severe enough to cause pain. Following this time lag, increased numbers of sweat glands became active in the heated area, and sweating spread to neighboring areas as temperature in these areas increased. In no experiments thus far carried out have the maximum number of functional sweat glands been activated by such a procedure. Provided the stimulating temperature is sufficiently high and applied to a large area, sweating may later appear on the control (unheated) arm as in figure 4. In a few instances however have as many glands been activated on the control arm as on the heated arm provided the innervation is bilaterally intact. Such observations would appear to rule out a rise in blood temperature at the sweat center as the important stimulus in contralateral sweating, since equal sweating on both sides might be expected if central temperature effects were responsible.

Kuno observed bilateral responses in secretion of sweat when one leg only was heated in a warm air bath (1930). As in our experiments, he noted much greater activity on the heated limb.

In patients with monolateral sympathectomy involving the upper extremity, we have observed sweating on the control (normally innervated, unheated) arm even though no sweating could be elicited simultaneously on the heated, sympathectomized arm. Sensory endings and pathways in these patients are intact, and the patient feels the locally applied heat.

Reflex activation of sweating on the contralateral side appears evident. Thus although pathways to the same side offer less resistance to central control of sweating, spread to the opposite side of the body is possible when a sufficiently strong and widespread stimulus is applied. This may be due to discharges from sweat centers in the brain or spinal cord, or to irradiation of afferent impulses in the cord. From evidence now available, the latter possibility seems the most probable explanation.

COMMENT. The results of these experiments demonstrate that secretion of the sweat gland is not a continuous process at ordinary room temperatures or even at such high temperatures as 35°C. Further, it is shown that varying numbers of glands may be activated in what appear to be phasic discharges over true secretory nerve fibers. Experiments have demonstrated that active sweating of individual glands is usually of very short duration (15 sec. or less) but may be of longer duration under suitable circumstances of stimulation. Although individual glands are thus active for periods of a few seconds only, a peak of sweating may be developed by progressively activating additional glands throughout a period of several minutes.

Further fragmentary evidence is available to indicate that vasomotor and sudomotor discharges do not necessarily occur simultaneously, thus supporting Darrow's studies (1929). Since the two systems are quite similar from a neurophysiologic viewpoint, one is reminded by the periodic activity of the sweat glands of the possibility (or probability) of a sweat center discharging periodically in a manner somewhat similar to that of the vasomotor center. This center, together with its auxiliary centers in the cord, would seem to be influenced by changes in blood temperature, impulses originating in the cerebral cortex, and

sensory pathways from the skin and viscera in a manner quite analogous to the vasomotor center.

SUMMARY

Between environmental temperatures of 20 to 35°C. sweat glands on the extensor surface of the forearm are phasically active showing alternating periods in which large and small numbers of glands are successively active in secreting onto the skin surface. The periodicity and the number of glands participating in each cycle of activity are not regular, but are influenced by environmental temperature, strong peripheral and visceral stimulation, and by psychic and emotional excitation. With the undisturbed subject resting in a comfortable environmental temperature, sweating is characterized by the sudden excitation of a number of sweat glands for a period of a few seconds followed by a period of little or no sweating activity by these glands. The secretory activity of individual glands under these conditions is thus relatively short, but activity may alternate between many glands in a given area and thus prolong the duration of a given sweating cycle. Extraneous excitation may also prolong a period of secretion, and cyclic activity may be superimposed upon the functional activity of large numbers of glands at high environmental temperatures.

When presented with a strong thermal stimulation, the sweat mechanism responds in two important stages. First, an increase in number of functional sweat glands following a few minutes after presentation of the stimulus. If this stimulus is sufficiently severe and prolonged, a second stage of sweating is marked by a large increase in output of the individual glands.

Simultaneous peaks and depressions in number of functional sweat glands on both forearms suggest the presence of a sweat center in the central nervous system discharging periodically and bilaterally to the sweat glands. When radiant heat is applied to one arm, sweating is likely to be localized in the area heated, but if the heating is sufficiently intense, bilateral sweating is observed, with greater numbers of active glands on the heated arm.

REFERENCES

- ADOLPH, E. F. *This Journal* **145**: 710, 1946.
DARROW, C. W. *This Journal* **88**: 219, 1929.
KUNO, Y. *Lancet* **1**: 912, 1930.
RANDALL, W. C. *Fed. Proc.* **5**: 84, 1946.
RICHTER, C. P. AND F. WHELAN. *J. Neurophysiol.* **6**: 191, 1943.

WATER AND SALT EXCHANGE DURING CHRONIC AND ACUTE DEHYDRATION IN THE DOG

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A considerable shifting of the water and salt content of tissues during periods of stress is known to occur. Nadal et al. (1941) suggested that in the human dehydration resulting from salt loss was followed by a decrease chiefly of extracellular fluid, while fasting and water deprivation caused a loss of both intracellular and extracellular fluid from the body organs. Hastings and Eichelberger (1937) have indicated that with injections of a balanced isotonic salt solution there was an increase in the extracellular phase with no change in the intracellular portion of the water in skeletal muscle. However, injections of an alkaline or acid salt solution was followed by changes in the water content of the intracellular, as well as the extracellular compartment of the musculature. The fluid exchange between striated muscle and plasma in fasting dogs with ample water intake has been shown by Mellors et al. (1942) to involve a loss of extracellular water, sodium and chloride from muscle tissue. Elkinton and Taffel (1942) have reported that prolonged water deprivation in the dog was followed by a loss of both intra- and extracellular water of the body accompanied by changes in the sodium content of blood and urine which suggested hypertonicity of the body fluids. It is known that the skin is able to lose considerable amounts of water during dehydration (Skelton, 1927; Underhill and Fisk, 1930; Hamilton and Schwartz, 1935). The chloride content of the skin, however, does not seem to be reduced under such conditions (Padtberg, 1910; Skelton, 1927).

The purpose of this investigation was to determine the changes in the water and chloride content of the skin and musculature during a period of chronic dehydration and recovery and to compare these changes with those occurring in these organs with acute dehydration as a result of hemorrhage.

MATERIAL AND METHODS. Thirteen dogs were used in this study. In 10 of these animals samples of skin were taken from the front and hind legs and from the back, and samples of the skeletal musculature in the same regions under normal conditions, and from symmetrically located areas after the period of dehydration without food or water for 6 to 7 days. At no time did the animals show any untoward symptoms or appear to suffer. All animals were operated upon under pentobarbital anesthesia. After the chronic dehydration period they were allowed to recover for 4-6 days during which no ill effects were noted. A third set of samples was then obtained from areas symmetrically located with respect to some of those supplying material at the height of dehydration. In four dogs, one of which had previously been used for a chronic dehydration experiment, the changes occurring in skin and muscle were determined after acute dehydration by hemorrhage of 9-20 grams/kgm. of body weight.

All tissues obtained were weighed immediately, placed in 25 cc. of purified ethyl alcohol for several days and desiccated at 103°C. \pm 1°C. until reaching a

TABLE 1

Effects of chronic and acute dehydration upon the water content of skin and muscle

DOG NO.	PER CENT OF WATER IN FAT-FREE SKIN				PER CENT OF WATER IN FAT-FREE MUSCLE			
	Control	After chronic dehydration	After recovery	After acute dehydration	Control	After chronic dehydration	After recovery	After acute dehydration
1	81.46	70.19			79.37	79.42		
2	74.74	77.18			78.03	78.04		
3	83.72	71.25			80.90	77.37		
4	75.38	74.22	73.53		78.35	75.64	76.94	
5	77.87	74.63	73.23		74.81	79.10	81.35	
6	76.60	74.93	71.51		79.19	79.40	79.85	
7	77.08	72.14	71.08		80.20	78.20	79.65	
8	75.40	71.76			80.26	79.52		
10	75.59	67.72			80.64	76.13		
13	74.95	72.88			80.26	78.19		
4	73.53			72.11	76.94			77.25
11	75.34			72.39	78.85			77.75
14	74.09			68.05	81.47			80.38
15	75.99			72.25	81.01			80.04

TABLE 2

Effects of chronic and acute dehydration upon the milligrams NaCl/100 cc. of water in skin and muscle

DOG NO.	SKIN				MUSCLE			
	Control	After chronic dehydration	After recovery	After acute dehydration	Control	After chronic dehydration	After recovery	After acute dehydration
1	149	556			212	184		
2	384	280			146	155		
3	249	464			119	199		
4	304	448	285		132	134		
5	270	447	444		239	137	196	
6	402	469	573		115	217	123	
7	297	352	491		180	178	220	
8	321	483			432	202		
10	335	517			154	162		
13	263	386			108	152		
4	285			239	132			98
11	324			406	112			121
14	339			387	163			114
15	333			409	175			96

constant weight. The fat was removed by ether extraction and the percentages of water and chloride were determined upon fat-free tissue, as these determinations have been shown to be more consistent (Peters et al., 1934; Manery et al.,

1938; Eichelberger, 1938; Eisele et al., 1945; DeBoer, 1946). The water content of the fat-free tissue was determined by desiccation after the removal of the fat. A total of 98 samples are here reported. In most cases more than one sample was obtained during an operation and the data represent average values. The chloride determinations were made by the Van Slyke-Sendrow method as modified by Sunderman and Williams (1933).

RESULTS. Dehydration by withholding food and water was followed by a greater loss of water from the skin than from the muscles of the body (table 1). Determinations of the water content of the fat-extracted skin before and after dehydration showed more than 4 per cent loss of water from this organ of the body. Control samples of skin gave an average water content of 77.3 per cent while after dehydration the samples averaged 72.7 per cent. Muscle tissue, on the other hand, decreased one per cent under the same circumstances, the average changes being from 79.2 per cent water to 78.1 per cent. Samples obtained after the animal had returned to its normal diet for 4-6 days showed that the skin remained depleted of its water supply with an average of 72.3 per cent water. After recovery muscle tissue with an average water content of 79.5 per cent was slightly higher than the control.

Acute dehydration resulted in a similar but smaller loss of water from the skin. The average water content of the samples of skin taken 2-4 hours after the hemorrhage was found to be 71.2 per cent as compared to 74.7 per cent for the control samples of these dogs. Muscle also showed less change in water content following acute dehydration, with an average water content of 78.9 per cent as compared with 79.6 per cent before hemorrhage.

The chloride content changed from an average of 297 mgm. NaCl per 100 cc. of water (50.0 m.Eq./l.) in the control samples of skin to 440 mgm. (75.3 m.Eq./l.) after chronic dehydration (table 2). The chloride content of the muscles deviated much less, the changes being from 184 mgm. (31.4 m.Eq./l.) in the control samples to 172 mgm. (29.4 m.Eq./l.) in the samples after dehydration. Following the return to the normal diet the chloride content of the skin remained at the level established by the chronic dehydration period with an average of 449 mgm. NaCl per 100 cc. (76.8 m.Eq./l.) of water found in the tissue. Muscle tissue, on the other hand, reverted toward the control condition with a value of 180 mgm. (30.8 m.Eq./l.). The chloride content of the skin after acute dehydration showed an increase from 320 mgm. (54.8 m.Eq./l.) in the control samples to 360 mgm. (61.6 m.Eq./l.) after dehydration. Muscle tissue showed a decrease in chlorides from 146 mgm. (24.9 m.Eq./l.) in the controls to 107 mgm. (18.4 m.Eq./l.) after acute dehydration.

In two dogs acute dehydration by hemorrhage was produced at the height of chronic dehydration. The water content of the skin and muscle did not deviate greatly from that of comparable samples taken before hemorrhage. The water content of the skin decreased less than 1 per cent and that of muscle less than 0.1 per cent. The chloride content of the skin under the above conditions showed an increase in one dog and a slight decrease in the other animal. The chloride content of the muscle decreased somewhat in both dogs.

DISCUSSION. Under the conditions of these experiments the amount of water lost from the skin was similar in both chronic and acute dehydration. The percentage loss of water from muscle was much less than from the integument. The changes noted in the chloride content appear significant in view of recent studies of fluid compartments and the distribution of electrolytes.

In normal muscle the amount of extracellular water has been determined by Eichelberger (1941) to be about 16 per cent of the total water of this tissue. Dean (1941) has shown that the chloride content of muscle is almost exclusively extracellular. On the basis of these investigations isotonic extracellular fluid of normal muscles of the dogs used in this study was calculated to be 1150 mgm. NaCl per 100 cc. If one further assumes that more than 90 per cent of the water lost during dehydration is extracellular, as indicated by Darrow and Yannet (1935a, b) the decrease in the chlorides and water of the musculature at the height of the dehydration period can be accounted for by loss of isotonic interstitial fluid. The changes in water and chlorides during recovery on the normal diet appear to be due to an influx of isotonic fluid into the musculature with a recovery of the extracellular compartment to the normal level.

Acute dehydration, however, was followed by a small loss of water from the muscles and a proportionately larger loss of NaCl. Mild hemorrhage at the height of the chronic dehydration period, although resulting in small loss of water, also was accompanied by a greater loss of chlorides than would be expected if isotonic fluid were lost from muscle tissue. These results are in accord with the work of Mellors et al. (1942) who found that the injection of 5 per cent glucose intraperitoneally and the removal of a similar amount of fluid after $4\frac{1}{2}$ hours was accompanied by a loss of extracellular electrolyte without loss of water from the musculature. They suggested that this was due to an increase in the intracellular compartment of skeletal muscle. The increase in NaCl in the water of the skin after chronic dehydration indicates not merely marked retention but actual storage as was suggested by Padtberg (1910). Although the musculature regained isotonic extracellular fluid during the recovery period the integument maintained this new water-salt balance. Assuming that the NaCl concentration of interstitial fluid of the integument equals that of the muscles, the increase in the chlorides in the skin following acute dehydration is proportional to the water lost from that organ.

The results indicate that during chronic dehydration muscle tissue shifts isotonic extracellular fluid to the circulatory system, whereas the skin loses chloride-free water and actually increases its chloride content. After hemorrhage, however, the isotonicity of the blood is maintained by obtaining water chiefly from the skin and chlorides from the musculature. The experiments involving acute dehydration at the height of chronic dehydration suggest that the conservation of fluid had reached a degree where tissues did not yield fluid even under the stress of rapid hemorrhage.

SUMMARY AND CONCLUSIONS

The changes in the water and chloride content in the skin and muscles were compared in dogs undergoing chronic and acute dehydration.

The changes in water and chloride of the skin with acute dehydration indicated that the fluid lost was devoid of its salt content. During chronic dehydration there appeared to be an actual accumulation of chlorides. This dehydrated condition persisted during a recovery period of 4-6 days.

The changes in water and chloride of the musculature suggested that isotonic extracellular fluid was lost during chronic dehydration and regained during recovery. During acute dehydration the muscle contributed considerable chlorides but little water.

Acute dehydration at the height of chronic dehydration indicated a conservation of fluid to a degree not appreciably changed by hemorrhage.

REFERENCES

- DARROW, D. C. AND H. YANNET. *J. Clin. Investigation* **14**: 266, 1935a.
J. Clin. Investigation **14**: 704, 1935b.
DEAN, R. B. *J. Biol. Chem.* **137**: 113, 1941.
DEBOER, B. *This Journal*, **147**: 49, 1946.
EICHEMBERGER, L. *J. Biol. Chem.* **122**: 323, 1938.
J. Biol. Chem. **138**: 583, 1941.
EISELE, C. W. AND L. EICHEMBERGER. *Proc. Soc. Exper. Biol. and Med.* **58**: 97, 1945.
ELKINTON, J. R. AND M. TAFFEL. *J. Clin. Investigation* **21**: 787, 1942.
HAMILTON, B. AND R. SCHWARTZ. *J. Biol. Chem.* **109**: 745, 1935.
HASTINGS, A. B. AND L. EICHEMBERGER. *J. Biol. Chem.* **117**: 73, 1937.
MANERY, J. F., I. L. DANIELSON AND A. B. HASTINGS. *J. Biol. Chem.* **124**: 359, 1938.
MELLORS, R. C., E. MUNTWYLER AND F. R. MAUTZ. *J. Biol. Chem.* **144**: 773, 1942.
NADAL, J. W., S. PEDERSON AND W. G. MADDOCK. *J. Clin. Investigation* **20**: 691, 1941.
PADTBERG, J. H. *Arch. f. exper. Path. u. Pharmacol.* **63**: 60, 1910.
PETERS, J. P. AND E. B. MAN. *J. Biol. Chem.* **107**: 23, 1934.
SKELTON, H. *Arch. Int. Med.* **40**: 140, 1927.
SUNDERMAN, F. W. AND P. WILLIAMS. *J. Biol. Chem.* **102**: 279, 1933.
UNDERHILL, F. P. AND M. E. FISK. *This Journal* **95**: 348, 1930.

HYPERCHROMIC ANEMIA PRODUCED BY CHOLINE OR ACETYLCHOLINE AND THE INDUCED REMISSION OF BOTH BY FOLIC ACID OR LIVER INJECTION. THE PROBABLE MECHANISM OF ACTION OF LIVER AND FOLIC ACID IN TREATMENT OF ANEMIA¹

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The production by choline feeding of a hyperchromic anemia in dogs which is responsive to liver injection has been reported previously by the author (1). More recently we have found that the administration of choline or carbamyl choline daily to dogs for a period of a month or more, causes changes in the central nervous system, some of which resemble those seen in human pernicious anemia (2).

This investigation was motivated by a desire to learn the mechanism by which liver injection causes remission of anemia. It was desired to see whether continued acetylcholine injections could cause anemia, and also to test the effect of folic acid upon choline anemia.

PROCEDURE. Eight normal dogs were used in these experiments. Their diet consisted of adequate amounts of Purina Dog Chow Checkers and water ad libitum. Control observations were made upon their blood until their normal basal erythrocyte counts and hemoglobin percentages seemed to be known. Then three of the dogs were given choline chloride in dilute solution by stomach tube in the amount of 200 mgm. twice daily. After 46 days of choline feeding, 2 of these dogs were given daily intramuscular injections of 2 mgm. of folic acid, additionally, while the third dog was kept as an anemic control animal for 14 days, after which time he was given Liver Injection (U.S.P.) at the rate of 2 units per day of a potent preparation.

The 5 other dogs each received 3 mgm. of acetylcholine bromide by subcutaneous injection twice daily. Two of these dogs were also given injections of 1 mgm. of physostigmine salicylate twice daily. After 47 days on this schedule, two of the dogs were given daily I.M. injections of 2 units of liver extract in addition to their anemia producing drugs; one dog was given 2 mgm. of folic acid, intramuscularly, in addition to his acetylcholine, and two animals were kept as anemic controls.

Erythrocyte counts and hemoglobin percentages (Hellige) were determined upon blood samples drawn from the saphenous veins of the dogs, while they were at rest, lying blindfolded upon a table. Reticulocyte percentages were determined upon dried films of blood stained with cresyl blue, and counterstained with Wright's stain.

¹ Research paper no. 588, journal series, Univ. of Arkansas.

Some estimations of acetylcholine activity were made upon extracts of blood serum by the use of the eserized rectus abdominis muscle of the frog. The method was essentially that of Chang and Gaddum (3), except that a muscle chamber of 5 cc. capacity was employed. Three cubic centimeters samples of serum were obtained from blood which was mixed with 0.3 per cent eserine in Ringer's solution (10 cc. of blood and 3 mgm. of eserine) immediately upon withdrawal from a vein. The serum was mixed with an equal volume of 10 per cent trichloroacetic acid solution, shaken, allowed to stand for 10 minutes, and filtered. To the filtrate (about 2.5 cc.), was added 2.5 cc. of frog Ringer solution. This mixture was then brought to a pH of about 6.6 by the addition of about 10 drops of 5 per cent sodium hydroxide solution, and more exact adjustment with weak HCl solutions. This neutralized mixture was then substituted for the Ringer solution in the muscle chamber enclosing the rectus muscle. If any response of the muscle was obtained, it was compared with the contractions elicited by standard acetylcholine solutions. The extracts were also tested on the non-eserized rectus, to make sure that any response was not due to potassium ion or to choline. Our test objects seemed sensitive enough to detect as little as 0.1 gamma of acetylcholine-like activity from 3 cc. of serum.

Cholinesterase activity of blood serum was determined by electrometric titration of the acetic acid liberated from a solution of 100 mgm. of acetylcholine bromide in 25 cc. of distilled water by 1 cc. of serum in 10 minutes. The method resembles that used by Goodman, Carlson, and Gilman (4) except that titrations were made at room temperature (22–26°C) and to a constant pH of 7.38. Activity was expressed as cubic centimeters of 0.01 N sodium hydroxide required to neutralize the acetic acid liberated over a period of 10 minutes.

RESULTS. The development of anemia in 3 choline-fed dogs is shown in figure 1, and the changes in erythrocyte numbers produced by acetylcholine in 5 dogs are shown in figure 2. Hemoglobin values were not reduced as greatly as the erythrocyte counts, and table 1 shows the status of the color indices in 8 dogs, both before the production of the anemias and at the time of the most marked anemias.

The two animals having the highest initial or pre-experimental red cell counts (fig. 2, solid and open dots) received 1 mgm. of physostigmine twice daily in addition to acetylcholine (3 mgm., b.i.d.). It is difficult to say whether or not the physostigmine potentiated the action of acetylcholine, but, nevertheless, it will be seen that all five "acetylcholine dogs" developed significant anemias (fig. 2).

Treatment of 2 choline-fed dogs was commenced on the 46th experimental day by the injection of 2 mgm. of folic acid², intramuscularly, per day. Reticulocyte percentages reached peaks of 3.4 and 4.2 on about the 8th day (fig. 3) at which time increases in red cell counts (fig. 1) became apparent. The erythrocyte numbers returned to normal in about 20 days in spite of continued choline administration. Hemoglobin was increased at a slower rate, as was true in our previous

² Synthetic L. Casei Factor was generously supplied by Lederle Laboratories, Inc., New York, N. Y.

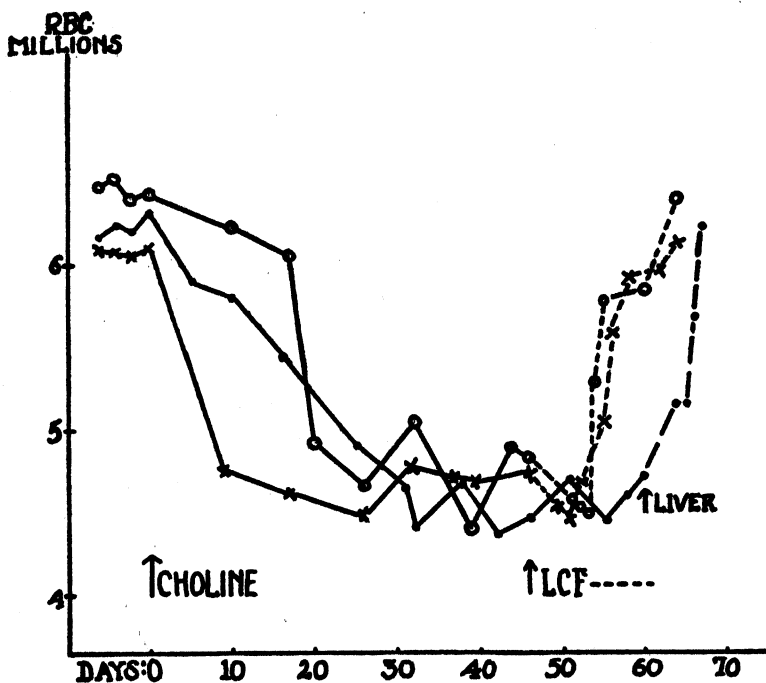


Fig. 1. Anemia induced by choline in 3 dogs, and treated by anti-anemic agents. Long dashes indicate period of liver treatment in one dog. Short dashes indicate period during which two dogs were given intramuscular injections of folic acid, daily, beginning at second arrow. (L.C.F. = L. Casei Factor.)

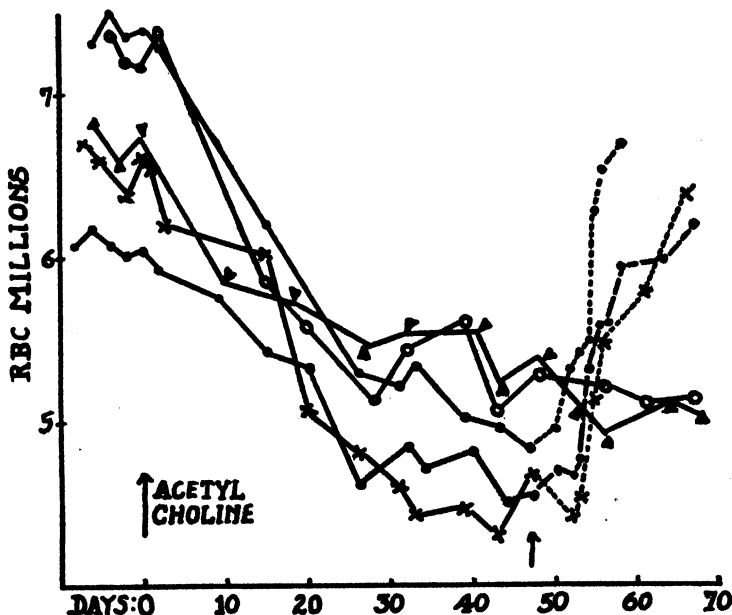


Fig. 2. Production of anemia in 5 dogs by acetylcholine, and induced remission of anemia in 3 animals. The two dogs having the highest initial red blood cell counts received physostigmine salicylate (1 mgm.) with each dose of acetylcholine, twice daily, throughout the experiment. At the second arrow, treatment of one dog with folic acid was started (long dashes), and treatment of two dogs with liver injections was commenced (short dashes).

work (1). The third choline-fed dog was kept as an anemic control animal for 14 days while the others were being treated, and was then given daily injections of liver extract at the rate of 2 units per day. It responded by showing a significant reticulocyte percentage peak of 4.0 per cent on the 6th day of treatment (fig. 3), and an apparent return to normal of his red cell count in seven days, even though choline feeding was continued.

Two of the anemic "acetylcholine" dogs were put on liver injection treatment on their 47th experimental day. They received 2 units of extract per day, and

TABLE 1

Color indices based upon an arbitrary normal erythrocyte number of 6 million and hemoglobin value of 13.1 grams per 100 cc of blood

	DOG NO.								AVERAGE
	1	2	3	4	5	6	7	8	
Pre-experimental.....	1.00	0.93	0.85	1.08	1.00	1.00	0.95	0.94	0.97
During anemia.....	1.20	1.11	1.00	1.22	1.16	1.22	1.08	1.29	1.16

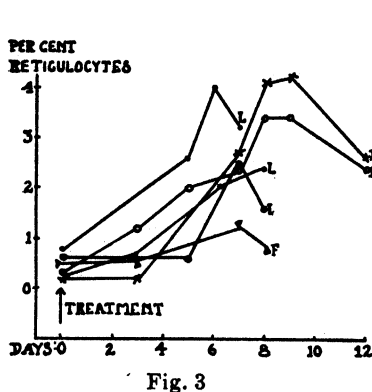


Fig. 3

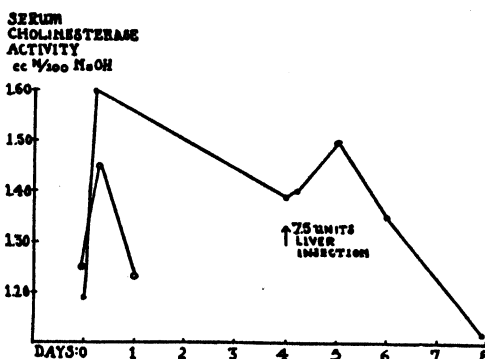


Fig. 4

Fig. 3. Reticulocyte responses to treatment of 6 anemic dogs. The three highest reticulocyte responses were obtained in the three dogs having choline-induced anemia. L after line indicates dog treated by liver injection. F indicates dogs treated with folic acid.

Fig. 4. Effect of orally administered folic acid upon the serum cholinesterase activity of two normal human subjects. Doses of 5 and 7.5 mgm. of folic acid were given at zero time. Four days later, one subject was given 7.5 units (0.5 cc.) of liver extract intramuscularly.

showed their maximal reticulocyte responses of about 2.5 per cent on the 7th and 8th days (fig. 3), and regeneration of red blood cells in one dog in 20 days (fig. 2), in spite of continued acetylcholine injections. One of these dogs, unfortunately, was killed in a dog fight, before his red cell count had been completely regenerated (fig. 2, short dashes and solid dots). He had received physostigmine in addition to acetylcholine.

Another dog of the acetylcholine series of animals (solid dots and long dashes, fig. 2) was treated by the daily injection of 2 mgm. of folic acid in addition to

acetylcholine. He responded with a reticulocytosis of 1.2 per cent on the 7th day of treatment (fig. 3, triangles) and a complete regeneration of red cells in about 20 days.

Acetylcholine-like activities in the blood sera of the 3 "choline" dogs are tabulated in table 2. These values determined on blood drawn at one and one-half hours after a choline dosage are in terms of acetylcholine, which the author believes they represent. The activity is potentiated by eserization of the frog's rectus muscle to the same extent that acetylcholine is, and is apparently not due to choline, potassium or chemical reaction. Table 2 shows that untreated choline anemia dogs have a higher acetylcholine activity in their serum

TABLE 2

Acetylcholine-like activity in the blood serum of dogs with choline-induced anemia, before and during treatment with folic acid or liver extract

Blood was drawn at one and one-half hours after feeding 200 mgm. of choline. Values are given in terms of gamma per 3 cc. of serum.

	DATE			
	4-26-46	4-29-46	5-7-46	5-18-46
Dog 1.....	0.4 anemic	0.3 anemic	0.4 anemic	0.25 L.E.T.
Dog 2.....	0.4 anemic	0.1 F.A.T.	0.1 F.A.T.	0.0 F.A.T.
Dog 3.....	0.45 anemic	0.0 F.A.T.	0.2 F.A.T.	0.0 F.A.T.

F.A.T. = folic acid treatment.
L.E.T. = liver extract treatment.
0.0 = less than 0.1 gamma.

TABLE 3

Cholinesterase activity in 1 cc. of serum, expressed as cubic centimeter of 0.01N sodium hydroxide required to neutralize the acetic acid liberated in a 10-minute period from a solution containing 100 mgm. of acetylcholine in 25 cc. (at 25°C and pH 7.38)

Dog 1.....	Anemic	0.17	Liver treatment	1.99
Dog 2.....	Folic acid treatment	0.92	Folic acid treatment	1.10
Dog 3.....	Folic acid treatment	0.40	Folic acid treatment	1.20

at 1½ hours after choline administration than they have later during treatment, or induced therapeutic remission. The decrease in this activity is particularly striking in the two dogs treated with folic acid, although it is also apparent in the one treated by liver (table 2).

Table 3 shows determinations of serum cholinesterase activity in our 3 "choline" dogs, which were not undertaken until rather late in the course of these experiments. In the first column it will be noted that untreated anemic dog 1 has a very low activity in comparison with the others which are under treatment with folic acid. One week later, when dog 1 was being treated by liver (column 2), all dogs show a higher cholinesterase activity, but the increase is most astounding in dog 1 who shows a 12-fold increase.

Table 4 shows cholinesterase activity in the blood sera of dogs during anemia and various stages of treatment, together with one human serum, and the *extent to which it is increased* by incubation for two and one-half hours with folic acid or liver extract. It was found that incubation of *serum alone* had no significant effect upon its cholinesterase activity. Folic acid or liver extract alone also had no cholinesterase activity. Nor did the addition of folic acid or liver to a mixture of substrate and serum have any significant effect.

It is shown in table 4 that, under the conditions of our experiment, the incubation of serum with liver or folic acid increased its cholinesterase activity significantly.

TABLE 4

Effect of incubation of blood serum with folic acid or liver extract at 37°C. for two and one-half hours upon its cholinesterase activity

Values are in terms of cubic centimeter of 0.01N sodium hydroxide, as in table 3.

INCUBATION MIXTURE	SERUM ALONE	INCUBATED WITH F.A. OR L.E.*	INCREASE
			%
1 cc. dog serum + 0.1 mgm. F.A.....	0.27	0.52	93
1 cc. dog serum + 0.1 mgm. F.A.....	0.40	0.63	57
1 cc. dog serum + 0.05 mgm. F.A.....	1.31	1.55	18
1 cc. dog serum + 0.1 mgm. F.A.....	1.31	1.67	27
1 cc. dog serum + 0.2 mgm. F.A.....	1.31	1.70	30
1 cc. dog serum + 0.1 mgm. F.A.....	2.20	2.63	19
1 cc. dog serum + 0.1 mgm. F.A.....	1.72	1.85	8
1 cc. dog serum + 0.75 μ L.E.....	0.80	1.05	31
1 cc. dog serum + 0.75 μ L.E.....	1.10	1.37	24
1 cc. dog serum + 0.16 μ L.E.....	1.72	1.88	9
0.5 cc. human serum + 0.16 μ L.E.....	1.16	1.33	15
0.5 cc. human serum + 0.1 mgm. F.A.....	1.14	1.30	14

* L.E. = liver extract.

F.A. = folic acid.

μ = U.S.P. unit.

Folic acid was given orally to 2 normal human subjects (fig. 4) and apparently increased the serum cholinesterase activity in each subject, within 5 hours following its administration. The increase amounted to 16 per cent in one subject and 33 per cent in the other. An intramuscular injection of 7.5 units of liver extract was given to one subject four days after he had taken 5 mgm. of folic acid. The effect of this procedure was slight, but perhaps, nevertheless significant.

DISCUSSION. Our results showing that acetylcholine can produce hyperchromic anemia should be of some significance, because of the fact that acetylcholine is liberated physiologically in the body and is considered a neuro-hormone or chemical mediator of nervous impulses or excitations. Furthermore, very little is known about the effects of a possibly occurring excess of naturally-liberated acetylcholine upon the intact body and its processes; or about diseases

that it could cause. It is also interesting that acetylcholine can affect blood formation, since we have already shown that the sympathico-mimetic hormone, epinephrine, is capable of producing polycythemia when injected daily over a period of time (5).

It is the author's opinion that choline-induced anemia is actually produced by *acetylcholine*, which might be synthesized from some of the administered choline. Some evidence favoring this opinion is found in the detection of acetylcholine-like activity in blood serum after choline administration (table 2).

We believe that acetylcholine produces anemia by a mechanism of action previously postulated for choline (1), namely, by producing vasodilatation and increased blood flow and oxygen supply to bone marrow—thus depressing erythrocytogenesis.

The discovery by Spies et al. (6) of the human anti-anemic properties of synthetic folic acid suggested the trial of this substance in our experimental anemias.

The fact that both folic acid and liver injection caused significant reticulocytosis and therapeutic remission of anemia in our experiments leads us to believe that these agents may probably be acting by the same mechanism of action in our dogs that they exert in human macrocytic anemias. At first we had thought that liver might possibly act by blocking the synthesis of acetylcholine from choline, but these experiments preclude that possibility.

It appears that both agents (folic acid and liver) act with some other substance (or are acted upon by other substances) to produce cholinesterase. We do not know whether the cholinesterase formed is of the "pseudo" or "true" variety, as yet.

Other suggestive evidence that liver extract contributed to an increase in cholinesterase activity, observed earlier but not considered significant at the time, was the finding that incubated mixtures of serum and liver extract, when mixed with definite amounts of acetylcholine at room temperature for exactly 5 minutes, *caused less vasodepressor action* in dogs following intravenous injection than acetylcholine which had been mixed with incubated serum alone.

It will perhaps be possible by refined incubation tests with a single suitable blood serum, to compare the potencies of various liver extracts with folic acid in regard to their ability to increase cholinesterase activity. We should mention, however, that some sera are not affected by incubation with these substances.

SUMMARY AND CONCLUSIONS

Significant hyperchromic anemia was produced in five dogs by the subcutaneous injection of 3 mgm. of acetylcholine twice daily. Two of the animals received 1 mgm. of physostigmine to reinforce each dose of acetylcholine.

Anemia was produced in 3 dogs by the oral administration of 200 mgm. of choline chloride twice daily.

The daily administration of folic acid to 2 dogs with choline anemia and 1 dog with anemia induced by acetylcholine caused a remission of their anemias, accompanied by early reticulocyte responses, in spite of continued choline or acetylcholine administration.

The daily injection of liver extract into 2 dogs with anemia produced by acetylcholine, and one dog with choline anemia, caused reticulocytosis and therapeutic remission of their anemias, in spite of continued administration of the anemia-producing agents.

Acetylcholine-like activity, of the order of 13 gamma per cent, was present in the serum of anemic dogs at one and one-half hours after the ingestion of 200 mgm. of choline chloride, but was diminished during treatment with folic acid or Liver Injection (U.S.P.). Serum cholinesterase activity, observed in one choline dog during anemia, was increased about 12-fold *during treatment* with liver.

The incubation of some blood sera (from dogs or men) with liver extract or with folic acid increased their cholinesterase activities.

Serum cholinesterase activity was increased in 2 human subjects by the ingestion of 5 and 7.5 mgm. of folic acid.

It is concluded that the important action of either folic acid or liver extract in the treatment of anemia probably is to increase the cholinesterase activity in the body.

REFERENCES

- (1) DAVIS, J. E. This Journal **142**: 402, 1944.
- (2) DAVIS, J. E. AND D. E. FLETCHER. To be published.
- (3) CHANG, H. C. AND J. H. GADDUM. J. Physiol. **79**: 255, 1933.
- (4) GOODMAN, CARLSON AND GILMAN. J. Pharmacol. and Exper. Therap. **66**: 15, 1939.
- (5) DAVIS, J. E. This Journal **137**: 699, 1942.
- (6) SPIES, T. D., C. F. VILTER, M. B. KOCH AND M. H. CALDWELL. South Med. J. **38**: 707, 1945.

THE RECOVERY OF INJECTED DYE AND THE YIELD OF BLOOD CELLS BY PERFUSION OF THE CARDIOVASCULAR SYSTEM IN BARBITALIZED DOGS¹

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Indirect methods for estimating the total volume of blood cells fall into three principal categories: 1. Determining the total intravascular hemoglobin from its percentage combination with foreign gases such as carbon monoxide, after administration of measured amounts of the latter. 2. Measuring the volume percentage of tagged cells after injecting a measured volume of the latter. 3. Estimating the plasma volume as the distribution volume of injected dye or other substance soluble in plasma, and calculating the cell volume from this value and the cell:plasma ratio in drawn blood. These methods might be expected, in theory, to yield somewhat different values. In practice, even larger deviations may result from errors in the major assumptions. The limiting assumption in the first method, for measuring intravascular hemoglobin, is that all of the administered gas combines with the hemoglobin of the red cells. The second method measures the total mobile cell volume only if the tagged cells behave exactly like normal cells within the circulation. The third method offers still larger possibilities for error, since any error in the plasma volume will carry over into the estimation. Furthermore, a true value for the mobile cell volume is obtained only if the circulating blood which is sampled has a cell:plasma ratio close to the mean for the entire vascular system. The present study was undertaken in an attempt to obtain a direct measurement of the cell volume, in the belief that reliable direct measurements are needed.

Exsanguination and post-mortem perfusion of the cardiovascular system may be expected to wash out the total volume of blood cells only if all the cells are mobile and accessible to perfusion. The Welcker washout procedure provides no evidence that these conditions prevail, since sequestered cells can be found in certain vascular channels even after hours of perfusion (Erlanger, 1921). To what extent these represent cells which are normally withheld from the circulation, and to what extent they become irreversibly sequestered as the animal is bled to death, is not known.

The possibility of irreversible sequestration during the washout may be greatly minimized, if not eliminated, by perfusing the living cardiovascular system with cell-free fluid (Harris, 1920; Amberson et al., 1935). In Amberson's procedure, blood is drawn at fixed intervals and replaced with an equal volume of cell-free fluid. The hematocrit declines by progressive dilution of the re-

¹ A partial report of these studies was made at the meeting of the American Physiological Society in Atlantic City, N. J., March 11-15, 1946.

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maining cells. The total washout is obtained by extrapolating the declining hematocrit to complete disappearance of cells. Since arterial pressure may be kept near its control level by using suitable replacement fluids, there is no reason to believe that blood will be trapped by cessation of flow in any channels which are normally open (Zweifach, Lee, Hyman and Chambers, 1944). If it can be shown that all the normally active portions of the circulation contribute to this type of washout, and if certain technical precautions are taken, the total extrapolated yields should represent the mobile cell volume. In the studies reported here, splenectomized dogs were subjected to this type of procedure. Since the spleen is the only demonstrable source from which red cells may be mobilized during hemorrhage under our experimental conditions (Overbey, 1946), the mobile cell volume should be equal in our studies to the circulating cell volume. Known amounts of the dye T-1824 were injected intravenously before starting the procedure, so as to obtain simultaneous values for the washout of dye and of cells.

In this type of procedure the calculation of the total yield is based upon the rate of decrease of dye and cells in the series of samples of drawn blood. If the infused fluid loses volume within the vascular system both cells and dye will decrease slowly, and if it gains volume they will decrease rapidly. Extrapolation to complete disappearance would yield larger values in the first instance than in the second, even though the total content were the same. The calculation is therefore meaningless unless data are obtained on the intravascular volume of the infused fluid.

METHODS. Normal dogs, fasted for 24 hours and anesthetized with sodium barbital, were splenectomized at least one hour before starting the blood volume studies. Plasma volume was estimated as the dilution volume of the dye T-1824 by a modification of the method of Gibson and Evans (1937). The dye was given, usually in a dose of 20 mgm., by rapid injection of a 0.1 per cent solution in saline into a femoral vein. Three blood samples were drawn from a femoral artery into oxalated centrifuge tubes at intervals of five minutes thereafter. They were centrifuged at 3000 r.p.m. for one hour in a centrifuge with head radius of 20 cm. Plasma dye concentrations were determined with the Coleman spectrophotometer, using as a reference undyed plasma from the same animal. The logarithm of dye concentration was plotted against time, a straight line was fitted to the three points, and the concentration at the time of injection was read at the intercept of this line with the 0 time axis. Plasma volume was computed from the formula: $P.V. = \frac{D}{c}$, where D is the amount of dye injected, in milligrams, and c is the plasma dye concentration at 0 time in mgm./ccm. A simultaneous value for the circulating cell volume was obtained as $C.V. = P.V. \times \frac{C}{\bar{P}}$, where $P.V.$ is the plasma volume as obtained above, and $\frac{C}{\bar{P}}$ is the volume ratio of packed cells to plasma in an arterial sample drawn just before injecting the dye. The packed cell volume was taken as $0.92 \times$ the volume read in the centrifuge tube to correct for trapped plasma (Chapin and Ross,

1942). The values obtained for circulating cell volume by this calculation are called hematocrit cell volumes in this report.

The washout was started as soon as possible after the completion of these initial determinations, usually within 20 minutes of the dye injection. Blood was drawn through an arterial bleeding cannula in a volume of 10 cc./kgm., oxalated, and transferred to a small stoppered flask. An equal volume of replacement fluid was injected as rapidly as possible into a femoral vein as soon as the withdrawal was completed, the entire procedure of bleeding and infusion usually requiring not more than 30 seconds. The bleedings and replacements were repeated at intervals of three minutes, measured from the start of the bleeding, the blood obtained on each bleeding being preserved in a separate flask. In most of the experiments 4 per cent gelatin solution³ was used as the replacement fluid, in a smaller number 0.9 per cent NaCl solution. With gelatin infusions mean arterial pressure, recorded from the other femoral artery with a mercury manometer, usually remained near its control level for ten to fifteen bleedings, to fall precipitously when the arterial hematocrit had been reduced to about 5 per cent. With saline infusions, pressure usually fell after the first four or five bleedings.

At the completion of the washout, aliquots were drawn from the series of flasks, after thorough shaking to resuspend the cells. The aliquot samples were centrifuged, the cell:plasma ratios determined, and the dye concentrations in the plasma measured, as in the case of the pre-washout samples. From these data the dye and cell yields on each bleeding were calculated.

Figure 1 shows the type of data obtained when the washout was continued to the death of the animal, using gelatin solution for replacement. The successive yields of both dye and cells declined at constant exponential rates throughout the perfusion. In the experiment shown in the figure, each yield of cells is approximately 0.825 times as large as the preceding yield, and each withdrawal of dye approximately 0.820 times the preceding value. For either dye or cells, therefore, the series of bleedings may be represented as: y , yr , yr^2 , yr^3 , ... $yr^{(n-1)}$, where y is the yield on the first bleeding, r has a value less than unity and n is the total number of bleedings. In all except the preliminary studies, the washout was discontinued after eight to ten bleedings, which were usually sufficient for accurate determination of r . For either cells or dye the total yield at complete disappearance from the drawn blood was calculated from the

formula: $Y = \frac{y}{1-r}$, where Y is the total yield, y the yield on the first bleeding, and r the constant ratio for the series. The value used for y in this calculation was read on the smoothed curve. All dye and cells drawn in sampling for the prewashout determinations were added to the washout yields, and are included in the total washout yields reported below.

No experiments were regarded as complete without data on the terminal plasma volume. The calculation given above for the total yield is valid only if

³ Gelatin solutions, grade P-20, were supplied by the Knox Gelatine Company, Camden, N. J.

the blood volume remains constant during the washout. Under these conditions, the terminal plasma volume should exceed the initial volume by an amount equal to the volume of cells actually withdrawn. Terminal plasma volume was measured by a new dye injection, using the three-sample technique described for the initial measurement. In about half the gelatin experiments, the terminal plasma volume was so close to the expected value that no correction in the total yields could be made. In the remainder, where evidence was found for expansion or contraction of the blood volume during the perfusion, a linear

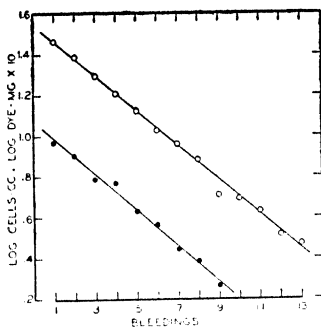


Fig. 1

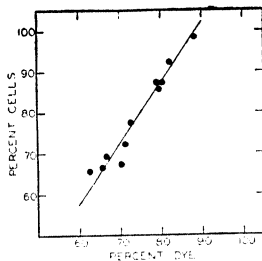


Fig. 2

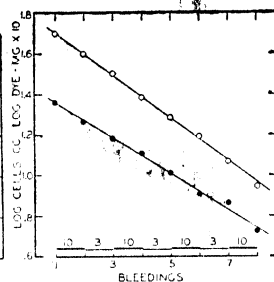


Fig. 3

Fig. 1. A washout of cells and of dye with 4 per cent gelatin solution. The cell yields are given as open circles, the dye yields as filled circles. Interval between bleedings three minutes, volume each bleeding 10 cc./kgm. Gelatin replacement 10 cc./kgm. immediately after each bleeding. Death occurred following the thirteenth bleeding, with arterial hematocrit 3.7 per cent. Dye concentrations were too low to be read with accuracy after the ninth bleeding.

Fig. 2. Relationship between percentage recovery of the hematocrit cell volume and percentage recovery of injected dye in gelatin perfusion experiments. The total yields were corrected for observed changes in blood volume during the perfusion. Three experiments of the type illustrated in figure 3 are included, along with the gelatin perfusions given

in table 1. The coefficient of correlation was obtained as: $\frac{\sum xy}{n-1} \cdot \frac{1}{\sigma_x \cdot \sigma_y} = 0.973$.

Fig. 3. A washout of cells and of dye with 4 per cent gelatin solution. Cell yields are given as open circles, dye yields as filled circles. Bleedings and replacements as in figure 1, except that the intervals between bleedings were alternately ten minutes and three minutes in duration. The numbers above the horizontal axis give the duration of each interval, in minutes.

change was assumed, and corrected values for Y were calculated from $r = r_a \left(1 + \frac{PV_i - PV_0 - C_b}{(n-1)(PV_0 + CV_0)} \right)$, where r_a is the observed constant ratio for the series of bleedings, PV_i is the terminal plasma volume, PV_0 the initial plasma volume, C_b the volume of cells actually withdrawn, n the number of bleedings, and CV_0 the initial hematocrit cell volume. A similar calculation of r from r_a on the assumption that observed changes in total blood volume were exponential rather than linear gave almost exactly the same values.

RESULTS. The essential data are given in table 1 and in figure 2. Since the

successive yields decline exponentially during the washout, each bleeding must contain a certain percentage of all the dye and of all the cells which are available at that time, and these percentages must remain constant throughout the perfusion. The data in table 1 on washout rates give the percentage yields per bleeding in all the experiments.

TABLE 1
Rate of yield and total yield for cells and dye

When the number of bleedings is n , the successive yields are $y, yr, yr^2, yr^3, \dots yr^{(n-1)}$. Rates of yield are given as $100 \times (1 - r)$. The total yield, extrapolated to complete disappearance (of dye or cells) is calculated as $\frac{y}{1 - r}$. The total yield of cells is given as a percentage of the initial hematocrit cell volume, and the total yield of dye as a percentage of the dye injected. In the gelatin perfusions changes in total blood volume during the washout were estimated from a redetermination of plasma volume at the end, and corrected values obtained for both the rate and the total yield (see text for nature of correction).

EXPT. NO.	RATE OF YIELD AS PERCENTAGE OF EXISTING TOTAL OBTAINED ON A SINGLE BLEEDING				TOTAL YIELD AT COMPLETE DISAPPEARANCE FROM DRAWN BLOOD. CELLS AS PER CENT OF HEMATOCRIT CELL VOLUME. DYE AS PER CENT OF AMOUNT INJECTED			
	Observed		Corrected		Observed		Corrected	
	Cells	Dye	Cells	Dye	Cells	Dye	Cells	Dye
Gelatin solution								
1	15.5	14.8	16.2	15.6	91.3	84.4	87.4	80.4
2	13.6	12.8	14.1	13.5	80.2	77.2	77.9	72.8
3	13.2	12.3	13.2	12.3	87.6	79.4	87.6	79.4
4	19.9	17.5	19.9	17.5	85.9	79.8	85.9	79.8
5	15.9	15.6	14.8	14.5	87.0	76.8	92.6	82.2
6	16.2	16.3	14.7	14.7	90.8	80.4	98.6	88.3
7	20.3	16.3	21.0	17.0	73.1	74.2	72.1	71.4
8	21.1	19.7	21.1	19.7	69.4	66.6	69.4	66.6
Ave.	16.96	15.66	16.88	15.60	83.16	77.35	83.94	77.61
Saline								
9	13.2	13.3			87.5	84.5		
10	14.7	11.2			62.1	78.4		
11	11.4	11.7			78.7	85.0		
12	13.1	14.1			89.1	74.3		
Ave.	13.10	12.58			79.35	80.55		

In the saline perfusions constant values for r were usually observed for only the first four or five bleedings. Coincident with the fall in mean arterial pressure which occurred at this time, there was usually a rather abrupt decrease in the value for r (cells and dye began to disappear more rapidly from drawn blood). The data in the table on saline washouts are based on the values ob-

served for r during the first portion of the perfusion, before arterial pressure had fallen. Since rapid failure of the circulation usually prevented measurement of the terminal plasma volume, the saline washouts cannot be corrected for changes in blood volume. The data obtained with saline perfusions are therefore of questionable value, except as controls for the gelatin experiments.

In the gelatin perfusions of table 1 it is apparent that each bleeding yields a somewhat larger percentage of the currently available cells than of the currently available dye. The two rates of yield, furthermore, appear to be closely correlated. The coefficient of correlation for the corrected rates in the table is 0.944. If the perfusion could be continued until both cells and dye disappear completely from the drawn blood, the cells would thus disappear first. This could mean that the cells occupy a smaller vascular compartment than the dye (see Smith, Arnold and Whipple, 1921; Fahreus, 1929); or it could mean that dye which was lost from the circulation before starting the washout is being returned to it during the perfusion (see Ferrebee, Leigh and Berliner, 1941). Dye loss from the circulation before starting the washout was estimated in our experiments from the decline in concentration observed during the pre-washout period, assuming that plasma volume remained constant during the period and subtracting from the total estimated loss the dye drawn in sampling. The average apparent dye loss within the animal was 1.15 mgm., with a range of 0.18 mgm. to 2.22 mgm. There is thus sufficient demonstrable dye loss before starting the washout to warrant serious consideration of the latter interpretation.

The total washouts in table 1 give the total (extrapolated) yield of cells as a percentage of the initial hematocrit cell volume, and the total yield of dye as a percentage of the dye injected. The yields were consistently less than 100 per cent, even when corrected for a change in total blood volume. The percentage yield of dye was consistently less than the percentage yield of cells. In figure 2 the percentage yield of cells is plotted against the percentage yield of injected dye. It is apparent from the figure that the two yields are closely correlated over a wide range of variation. The nearly perfect correlation of 0.973 is obtained for the two variables in the figure.

Incomplete recovery of the injected dye is not surprising, since dye disappears from the circulation with time, and extravascular dye would probably not be washed out completely by our procedures. A fractional washout of the hematocrit cell volume was also expected, since there is reason for believing that the true cell volume is somewhat less than this value (Hahn, Balfour, Ross, Bale and Whipple, 1941; Stead and Ebert, 1941; Lawson and Rehm, 1945). The unexpected finding is the close correlation between the two fractional washouts. If our washout procedures can be shown to be valid for measurement of the total content of the vascular system, it is apparent that the error in the hematocrit cell volume is in some way closely correlated with the amount of dye lost from the circulation.

Since the gelatin solutions used in most of the experiments have a colloid osmotic pressure of about 30 mm. Hg (Lawson and Rehm, 1943), they might be expected to gain volume within the circulation. Although our measurement

of terminal plasma volume permits a correction of the yields for expansion of the blood volume, it must be admitted that the correction may be inadequate. Under these conditions, gelatin perfusions might be expected to yield, by our methods, less than the total content of both dye and cells. Conversely, since salt solution could hardly gain volume within the circulation, and would probably lose volume, saline perfusions might be expected to yield values in excess of the total content. That errors from this source are of little importance in our experiments is suggested by comparing the uncorrected data obtained with the two types of perfusion in table 1.

Less than the total content will be obtained by our methods if blood becomes segregated during the perfusion. Although trapping of blood does not occur in our experiments simply as a result of a fall in arterial pressure, other mechanisms could operate. Gelatin solutions cause pseudo-agglutination of red cells within the circulation (Ivy, Greengard, Stein, Grodins and Dutton, 1943), and, although embolism has not been demonstrated, the possibility of its occurrence cannot be dismissed, especially when the circulation is disturbed by progressively increasing asphyxia as in our experiments. These factors could be eliminated by using whole blood as the perfusion fluid. In the experiment of table 2, part A, a washout of dye was done by perfusing with undyed, freshly drawn, heparinized whole blood. The total dye yield was 75 per cent of the injected dye, very close to the average yield obtained with gelatin. Comparison of the terminal and the initial hematocrit cell volume provides empirical evidence, in this experiment, against trapping of cells during the perfusion. A special attempt was made, in four animals, to flush out at the end of the experiment blood which might have become segregated during gelatin perfusion. Typical results are shown in table 2, part B. After the washout was completed, and terminal values obtained for the hematocrit cell volume and the dye remaining in the circulation, 20 cc./kgm. of gelatin solution were infused, and these values redetermined. As shown in the table, such expansion of the blood volume did not result in increasing either the total circulating dye or the total circulating cells as measured by the hematocrit cell volume. If blood becomes segregated during gelatin perfusion, therefore, it appears incapable of being flushed into the circulation by these methods.

Since the interval between an injection of the perfusion fluid and the subsequent bleeding was something less than three minutes in our experiments, it seemed not unlikely that our results were attributable to incomplete mixing. If this interval is too short to permit the injected fluid to pervade all portions of the vascular system, the drawn blood will contain disproportionately large amounts of the perfusion fluid. The washout yields, under these conditions, would represent only the cells and the dye in the more rapidly moving vascular circuits. This possibility was examined in four experiments of the type illustrated in figure 3. The intervals between infusion and subsequent bleeding in these experiments were made alternately three and ten minutes long. If the length of the intervals is not a significant factor, it should be possible to ignore the time scale in plotting the results, as has been done in the figure.

The successive yields, plotted in this way, fit exponential curves as well as do those obtained at regular intervals. If the length of the intervals were a limiting factor for our data, the yields obtained at the end of the shorter intervals would be expected to lie below the average exponential curve, those at the end of the longer intervals above it. The length of the interval, between three and ten minutes, does not appear from these experiments to be an important factor. In the experiment illustrated in the figure the total cell washout was

TABLE 2

	Cells	Dye
A. A washout of dye with heparinized whole blood. Weight of animal 9.2 kgm.		
	cc.	mgm.
Initial values (hematocrit cell volume, dye injected) . . .	398	15.0
Total yield (1)		11.18
Actual withdrawal during perfusion	395	8.40
Replaced during perfusion	308	0.00
Net actual loss during perfusion	87	8.40
Terminal values (2)	319	4.21
Terminal values plus net actual loss	406	12.61
B. An attempt to flush additional cells and dye into circulation by injecting large volumes of gelatin solution at end of perfusion. Weight of animal 11.6 kgm.		
Initial values (hematocrit cell volume, dye injected) . .	547	20.0
Total yield (3)	479	15.86
Actual withdrawal during perfusion	350	11.06
Terminal values (2)	162	5.74 (+10.0)
Values 10 minutes after injection of 235 cc. gelatin solution (4)	162	13.86

(1) Contains correction of -0.11 mgm. for 70 cc. decrease in blood volume during perfusion.

(2) Hematocrit cell volume by injection of 10 mgm. dye at end of perfusion. Total circulating dye as terminal plasma volume \times plasma dye concentration before new dye injection.

(3) Measured change in blood volume during perfusion $+14$ cc., no correction applied.

(4) By re-injecting dye after the gelatin had been given. Terminal plasma volume before gelatin injection 990 cc., after gelatin injection 1205 cc. Total circulating dye before the gelatin injection should be $5.74 + 10.0$ mgm.

67 per cent of the hematocrit cell volume, and the dye washout was 66 per cent of the dye injected.

DISCUSSION. It may be assumed that perfusion under physiological conditions will wash out only those cells which are freely movable within the vascular system. There is no reason for believing that blood cells which are fixed in either the blood forming or the blood destroying tissues will be dislodged. The method should, therefore, yield somewhat less than the total volume of cells. If the movable cells are uniformly distributed throughout the entire plasma volume, the washout of cells should agree with the hematocrit cell volume, as-

suming that both measurements are technically acceptable. If, however, any appreciable volume of plasma is cell-free (Smith, Arnold and Whipple, 1921; Fahreus, 1929), the hematocrit cell volume should exceed the washout. The percentage error in the hematocrit cell volume, under these conditions, should be exactly equal to the percentage of the total plasma volume which is free of cells. Hahn, Balfour, Ross, Bale and Whipple (1941) found the circulating cell volume as measured by injecting radioactive tagged cells to be about 75 per cent of the hematocrit cell volume. An error of similar magnitude in the hematocrit cell volume is suggested by its excessive change when cells are withdrawn or injected (Smith, Arnold and Whipple, loc. cit.; Stead and Ebert, 1941; Hahn and Bale, 1942; Lawson and Rehm, 1945). If the true circulating cell volume be taken as approximately 75 per cent of the hematocrit cell volume on the strength of these data, our washout of cells seems to be complete, since our average yield was 83.94 per cent of the hematocrit value.

Complete recovery of the injected dye, in these experiments, would provide additional assurance that all parts of the vascular system are reached by the perfusion. Since the average recovery of dye was only 77.61 per cent, there is no direct proof that all parts of the system have been washed out. If our perfusions are incomplete because trapping occurs during the washout, the trapping appears to be irreversible, and not to be prevented by using whole blood for the perfusion fluid. Furthermore, trapping of blood during the washout would hardly be expected to progress at such a constant rate as to permit the exponentially declining yields which were observed. If our perfusions are incomplete because too little time is allowed for the added fluid to circulate, the circulation rate through the unperfused channels must be very slow. Increasing the time for circulatory mixing of the added fluid from three minutes to ten minutes was without effect on the yields.

In the absence of direct proof that our methods yield true values for total vascular content, the following interpretations of the closely correlated percentage washouts of dye and cells are possible: 1. Only a portion of the vascular system is perfused. This portion contains approximately equal percentages of the injected dye and of the hematocrit cell volume. *a.* If the hematocrit cell volume represents the true cell volume (Root, Roughton and Gregersen, 1945), the unperfused vascular circuits must contain cells and plasma in the same relative proportions as are found in the perfused circuits. *b.* If the washout cell volume represents the true value, the unperfused circuits must contain no mobile cells. 2. The entire vascular system is perfused. The correlation between dye and cell washout is due to the fact that the hematocrit cell volume is calculated from an assumed value for total circulating dye. The percentage error in the hematocrit cell volume is thus equal to the percentage of the injected dye which disappears from the circulation undetected, i.e., between the time of dye injection and the first sampling. The hematocrit cell volume cannot be a true value, within this interpretation.

The latter interpretation, which assumes a consistent error in our measurements of plasma volume, cannot be dismissed without serious consideration.

Plasma volume is calculated as $\frac{D}{c}$, where D is the amount of dye injected, and c is the extrapolated value obtained for plasma dye concentration at the instant of injection. The present methods of extrapolation are valid only if dye leaves the vascular system at a constant rate following its injection. Reliable data on dye loss during the first few minutes are difficult to obtain, since mixing within the circulation should have the same effect on the concentration in drawn plasma as dye loss from the vascular system. The present extrapolation is based on the assumption that rapidly falling dye concentrations such as may be observed in successive samples drawn during the first five minutes, are due to mixing (Gibson and Evans, 1937). If there is excessive loss of dye from the plasma during this period, as has been suggested by Cruikshank and Whitfield (1945), the plasma volume should be calculated as $\frac{D-d}{c}$, where d is the total excessive (i.e., undetected) loss during the first five minutes. If assurance could be given that our washout yields represent total vascular content, it would be possible to obtain a value for d . The washout of cells, under these conditions, could be formulated as $Y_c = \frac{(D-d)}{c} \times \frac{C}{\bar{P}}$, where Y_c is the cell washout, and $\frac{C}{\bar{P}}$ is the cell:plasma ratio in arterial blood. Solution of this equation for $(D-d)$ in our experiments yields corrected values for circulating dye ranging from 13.88 to 19.70 mgm., with an average of 16.38 mgm. Since 20 mgm. of dye were injected, this would mean an average excessive loss, d , of 18.13 per cent of the injected dye during the first five minutes. Our washout yields of dye vary from 88.8 to 99.0 per cent of the corrected values, with an average of 94.0 per cent.

SUMMARY

Barbitalized, splenectomized dogs were given intravenous injections of the dye T-1824, and about 20 minutes later perfusion of the cardiovascular system with 4 per cent gelatin solution was started. The perfusion was done by withdrawing 10 cc./kgm. of blood from an artery, and immediately injecting 10 cc./kgm. of gelatin solution intravenously, repeating the bleeding and replacement every three minutes. The successive yields of dye and of blood cells declined at constant exponential rates, which were extrapolated to obtain the total washouts. The cells usually disappeared more rapidly than the dye from the drawn blood. The total washout of dye averaged 77.61 per cent of the dye which had been injected, and the total washout of cells averaged 83.94 per cent of the initial hematocrit cell volume (calculated as the product of plasma volume by the arterial cell/plasma ratio). The percentage recovery of dye varied widely, from about 62 to about 88 per cent, and the percentage washout of the hematocrit cell volume similarly varied from about 66 to about 99 per cent. There is a nearly perfect correlation (coefficient = 0.973) between the percentage recovery of dye and the percentage washout of the hematocrit cell volume.

Alternative interpretations of the data are: 1. The washout method used in this study yields only a fraction of the total vascular content. 2. The ratio

between the true circulating cell volume and the hematocrit cell volume depends upon the percentage of the injected dye which leaves the vascular system undetected, during the so-called "mixing period."

REFERENCES

- AMBERSON, W. R., J. FLEXNER, F. R. STEGGERDA, A. G. MULDER, M. J. TENDLER, D. S. PANKRATZ AND E. P. LAUG. *J. Cell. and Comp. Physiol.* **5**: 359, 1935.
- CHAPIN, M. A. AND J. F. ROSS. *This Journal* **137**: 447, 1942.
- CRUIKSHANK, E. W. H. AND I. C. WHITFIELD. *J. Physiol.* **104**: 52, 1945.
- ERLANGER, J. *Physiol. Rev.* **1**: 177, 1921.
- FAHREUS, R. *Physiol. Rev.* **9**: 241, 1929.
- FERREBEE, J. W., O. C. LEIGH AND R. W. BERLINER. *Proc. Soc. Exper. Biol. and Med.* **46**: 549, 1941.
- GIBSON, J. G. AND W. A. EVANS. *J. Clin. Investigation* **16**: 301, 1937.
- HAHN, P. F. AND W. F. BALE. *This Journal* **136**: 314, 1942.
- HAHN, P. F., W. M. BALFOUR, J. F. ROSS, W. F. BALE AND G. H. WHIPPLE. *Science* **93**: 87, 1941.
- HARRIS, D. T. *Brit. J. Exper. Pathol.* **1**: 142, 1920.
- IVY, A. C., H. GREENGARD, I. F. STEIN, G. S. GRODINS AND D. F. DUTTON. *Surg., Gynec. and Obst.* **76**: 85, 1943.
- LAWSON, H. AND W. S. REHM. *This Journal* **144**: 199, 1945. *Ibid.* **140**: 431, 1943.
- OVERBEY, D. T. *Fed. Proc.* **5**: 78, 1946.
- ROOT, W. S., F. J. W. ROUGHTON AND M. I. GREGERSEN. *Fed. Proc.* **4**: 60, 1945.
- SMITH, H. P., H. R. ARNOLD AND G. H. WHIPPLE. *This Journal* **56**: 336, 1921.
- STEAD, E. A. AND R. V. EBERT. *This Journal* **132**: 411, 1941.
- ZWEIFACH, B. W., R. E. LEE, C. HYMAN AND R. CHAMBERS. *Ann. Surg.* **120**: 232, 1944.

THE INFLUENCE OF CALORIC RESTRICTION ON PLASMA PROTEIN, HEMOGLOBIN AND HEMATOCRIT VALUES IN THE RAT¹

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Numerous investigators have reported that plasma protein, hemoglobin and hematocrit values are lower in the very young growing rat than in the adult animal (1-6). Similar results have been noted in man (7). However, sex differences in the blood picture are absent in the rat (8) and, in this respect, the results vary from man.

It is of considerable interest to determine whether the variations in the blood constituents are a function of age or of size. Such information can of course be obtained only in experiments where the caloric intake is restricted. There are little data in the literature on the influence of dietary restriction on the blood components of the young rat. Albanese *et al.* (4) list the results they obtained with 5 rats, weighing 60-133 grams, in which the food intake was limited to 5 grams daily. After 26 to 236 days when the final weights were 87-141 grams, an essentially normal blood picture was observed.

METHODS. Weanling rats, 21 days old, in groups of 50, were placed on each of six diets of varying fat content (table 1; 60c, 61, 62, 63, 64, and stock diet) in individual cages. These rats were fed at a caloric level which was just sufficient to insure survival for 12 weeks, with minimal weight gain. Similar groups of rats, 5 to a cage, were placed on each of 7 diets (60a, 60b, 61, 62, 63, 64, and stock diet) and were fed *ad lib.* for 12 weeks. The composition of these diets is given in table 1.

At the end of the 12 week feeding period, heparinized blood was collected from 4 to 6 rats in each group by heart puncture. Ether anesthesia was used. The specific gravity of whole blood and of plasma was determined by the copper sulfate method of Phillips *et al.* (10) since this method is very rapid and requires minimal amounts of blood. Plasma protein values were calculated by the use of the nomogram of the authors. However, values for the hematocrit calculated by this nomogram did not agree with the values obtained by direct hematocrit determination. Hematocrit values were therefore calculated by the use of Ashworth and Tigertt's formulation (11):

$$H = 100 \times \frac{G_B - G_p}{G_C - G_p} \quad (I)$$

¹ This report represents part of a study on the effects of diets of varying fat contents on rat growth and performance. We are indebted to the Committee on Food Research, Office of the Quartermaster General, for a grant for the studies where restricted diets were employed. The results on the *ad libitum* feedings were supported by a grant from The Best Foods, Inc.

where G_B is the specific gravity of whole blood G_C is the specific gravity of centrifuged cell, G_P is the specific gravity of plasma, and H is the hematocrit. If H , G_B and G_P are measured, G_C can be calculated according to equation II:

$$G_C = \frac{100 (G_B - G_P) + H G_P}{H} \quad (\text{II})$$

TABLE 1

Diets

<i>Stock diet:</i>	<i>Per cent</i>		<i>per cent</i>
Whole wheat, ground.....	34.0	Alfalfa meal.....	4.0
Oats, steel cut, ground.....	34.0	Sodium chloride.....	0.5
Skim milk powder.....	15.0	Calcium carbonate.....	0.5
Cottonseed oil.....	10.0	Strain G yeast.....	2.0
(containing vitamin A 1600 IU vitamin D 160 IU)			

Synthetic diets:

COMPONENTS	DIET NUMBER						
	60a	60b	60c	61	62	63	64
Sucrose.....	67	67	65	61	54	40	12
Casein (vitamin test).....	25	25	25.5				
Casein (commercial).....				27	28	31	37.5
Cottonseed oil.....				4	9	19	39
Cellulose.....	3	3	3	3	3.5	4	4.5
Salt mixture.....	4	4	4	4	4.5	5	6
Fat-soluble vitamins*.....	1						
Fat-soluble vitamins†.....		1	1				
Fat-soluble vitamins‡.....				1	1	1	1
Methyl linolate.....			1.5				
Water-soluble vitamins§.....	0.16	0.16	0.16	0.17	0.18	0.20	0.24

* Tocopherol, 1 gram, carotene, 40 mgm., vitamin D₂, 1 mgm. made up to 200 grams with ethyl laurate. Crystalline vitamin D₂ was kindly furnished by the Winthrop Chemical Company.

† Fat-soluble vitamins as above made up to 200 grams with methyl linolate.

‡ Fat-soluble vitamins as above made up to 200 grams with cottonseed oil.

§ Choline, 75 grams, thiamine, 1 gram, riboflavin, 1 gram, pyridoxine, 1 gram, calcium pantothenate, 2 grams, folic acid, 0.25 gram. The choline, thiamine, riboflavin and calcium pantothenate were donated by Merch and Co., and the folic acid by Lederle and Co.

Blood samples were obtained from 47 rats of various ages fed all types of diets. Hematocrits were determined on these samples, after centrifuging for one hour at 3000 R.P.M. in an International centrifuge in which the centers of the resting hematocrit tubes were 13 cm. from the axis. G_B and G_P were determined by the copper sulfate method of Phillips *et al.* (10). The G_C value for rat blood calculated by equation II from these data was 1.0911. Hematocrit values for the bloods obtained from rats on the experimental diets given in table 1 were then calculated by the use of equation III:

$$H = 100 \times \frac{G_B - G_p}{1.0911 - G_p} \quad (\text{III})$$

The standard deviation of the calculated from the observed hematocrit value, using 1.0911 for G_c , was 5.9 per cent.

Hemoglobin values were calculated by the use of equation IV (10):

$$\text{Gram hemoglobin per 100 cc. of blood} = 33.9 \times \frac{G_B - G_p}{1.0911 - G_p} \quad (\text{IV})$$

TABLE 2

Relation of food intake to plasma protein, hematocrit and hemoglobin in 15 week old rats

GRAMS FED IN 48 HRS.	DIET	SEX	NO. OF RATS	AVERAGE WEIGHT AT 15 WKS.	PLASMA PROTEIN	HEMATOCRIT	HEMOGLOBIN
				grams	gm./100 cc.	per cent	gm./100 cc.
6.3	60c	M	5	54.6	4.85	39.0	13.2
6.3	60c	F	6	55.2	4.68	39.9	13.5
6.2	61	M	5	61.8	4.66	37.5	12.7
6.2	61	F	3	49.6	4.25	38.5	13.1
5.8	62	M	4	57.6	4.57	36.4	12.3
5.8	62	F	3	55.3	4.64	37.5	13.0
5.3	63	M	6	62.4	4.73	37.1	12.6
5.3	63	F	5	58.2	4.51	36.8	12.5
4.4	64	M	3	64.3	4.66	35.5	12.0
4.4	64	F	5	57.2	4.60	37.1	12.6
6.0	S	M	2	56.5	4.93	33.8	11.6
6.0	S	F	4	54.0	4.74	40.1	13.6
<i>Ad lib.</i>	60a	M	5	199	5.58	46.5	15.7
	60a	F	3	152	5.74	45.8	15.6
	60b	M	6	210	5.79	47.1	16.0
	60b	F	5	151	5.68	43.1	14.6
	61	M	4	281	5.84	46.8	15.9
	61	F	4	193	5.67	45.1	15.3
	62	M	4	265	5.56	46.2	15.7
	62	F	4	201	5.62	41.8	14.2
	63	M	5	297	5.28	45.1	15.3
	63	F	3	204	5.21	42.9	14.6
	64	M	4	281	5.39	44.0	15.0
	64	F	5	197	5.71	44.2	15.0
	S	M	4	249	4.85	40.6	13.8
	S	F	5	188	5.20	41.6	14.1

RESULTS. Rats which were restricted in their food intake showed only slight increases in weight in the 12 week period: average starting weight, males, 33.8 grams, females, 35.8 grams; average weight gain, males, 21.7 grams, females, 19.1 grams. On the other hand, rats fed *ad libitum* gained progressively and at an essentially normal rate; average starting weight, males, 35.5 grams, females, 35.5 grams; average weight gain, males, 217.9 grams, females, 141.8 grams.

The data on plasma protein, hemoglobin and the hematocrit for the rats on *ad lib.* feeding and on the restricted diets are summarized in table 2.

There are marked differences in the plasma protein, hematocrit and hemoglobin values between the rats which were on a restricted food intake and those fed *ad libitum*. In all cases the values are consistently lower in the blood of rats on the restricted intake. No striking differences are apparent between the diets of different fat content, nor between male and female rats.

Table 3 presents similar data obtained from very young control rats, averaging 52.1 grams in weight, and from older rats, averaging 256 grams in weight, fed stock diet *ad lib.* The plasma protein, hematocrit, and hemoglobin values of the very young rats are very similar to the values observed in 15 week old rats, which have been so restricted in their food intake that they gained roughly only 10 per cent of the normal weight increase for this period.

These data confirm the results of other investigators (1-6) that the level of hemoglobin, plasma protein and the hematocrit are considerably lower in the young rat on an adequate diet than in the adult animal. When the caloric level of the diet was kept at a minimum value over a 12 week period, sufficient to per-

TABLE 3
*Plasma protein, hematocrit and hemoglobin in normal male and female rats**

WEIGHT	NUMBER OF RATS	PLASMA PROTEIN	HEMATOCRIT	HEMOGLOBIN
<i>grams</i>		<i>gm./100 cc.</i>	<i>per cent</i>	<i>gm./100 cc.</i>
52	13	4.15	35.9	12.2
256	16	6.29	43.8	14.9

* Fed stock diet *ad lib.*

mit the animals to live but inadequate for growth, the hemoglobin, plasma protein, and hematocrit values corresponded with those found in rats of the same size rather than the same age. Our results indicate, therefore, that one or more factors other than age determine the level of these blood components. The results of Albanese *et al.* (4) who also restricted the dietary intake in young rats are not directly comparable with ours since the final weights of their animals varied between 97 and 141 grams while the final weights of our rats on restricted diets averaged 52 grams. It is not clear, however, whether or not the low hemoglobin and plasma proteins found in the rats on the restricted diets are related directly to their size or indirectly to some other phenomenon such as their degree of maturation. In no case had maturation of the females (as judged by opening of the vagina) occurred in the rats on the restricted diets within 12 weeks after weaning. With the rats on the *ad libitum* feeding the average maturation time was about four weeks after weaning.

SUMMARY

Equations for the calculation of hemoglobin, plasma proteins and hematocrit in rat blood, based on the procedure of Phillips *et al.*, are given.

In confirmation of other investigators, it is demonstrated that the hemoglobin, plasma protein and hematocrit values are lower in very young rats than in the adult.

When weanling rats are maintained on diets sufficiently restricted in calories to prevent appreciable growth over a 12 week period, the values for hemoglobin, plasma protein and hematocrit correspond with those of rats of the same size rather than of the same age.

REFERENCES

- (1) SURE, B., M. C. KIK AND D. J. WALKER. *J. Nutrition* **1**: 299, 1929.
- (2) MITCHELL, H. S. AND L. MILLER. *This Journal* **98**: 311, 1931.
- (3) WACHTEL, L. W., C. A. ELVEHJEM AND E. B. HART. *This Journal* **140**: 72, 1943.
- (4) ALBANESE, A. A., L. E. HOLT, C. N. KAJDI AND J. E. FRANKSTON. *J. Biol. Chem.* **148**: 299, 1943.
- (5) METCOFF, J. AND C. B. FAVOUR. *This Journal* **142**: 94, 1944.
- (6) METCOFF, J., C. B. FAVOUR AND F. J. STARE. *J. Clin. Investigation* **24**: 82, 1945.
- (7) PETERS, J. P. AND D. D. VAN SLYKE. *Quantitative chemical chemistry*. Vol. I. Williams and Wilkins Co., Baltimore, p. 544.
- (8) WINTROBE, M. M., H. B. SHUMACKER, JR. AND W. J. SCHMIDT. *This Journal* **114**: 502, 1936.
- (9) PHILLIPS, R. A., D. D. VAN SLYKE, V. P. DOLE, K. EMERSON, JR., P. B. HAMILTON AND R. M. ARCHIBALD. Copper sulfate method for measuring specific gravities of whole blood and plasma. Josiah Macy, Jr. Foundation, New York, February 1945.
- (10) ASHWORTH, C. T. AND W. D. TIGERTT. *J. Lab. and Clin. Med.* **26**: 1545, 1940-41.

THE EFFECT OF METHYL XANTHINES ON THE FORMATION OF UREA IN RABBITS

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In 1916 Benedict (1) showed that caffeine given in fairly large doses over several days caused nitrogen retention in man. The total nitrogen excretion dropped to about 60 per cent of that in the control period and when caffeine was discontinued, it gradually returned to normal. Benedict did not determine urea excretion but it is probable that it decreased to the same extent as the total nitrogen. A possible explanation for this was obtained when it was shown that caffeine, theophylline and theobromine inhibit urea formation from ammonium salts by liver slices (2). Also when rabbits are fed on an acid producing diet of oats and are given ammonium chloride, the extra urea excretion caused by the latter is inhibited if caffeine is administered simultaneously (3). This observation raised several questions. Will caffeine and the other methyl xanthines decrease urea excretion of rabbits on a more normal diet without the administration of ammonium chloride? Will the total nitrogen fall to the same extent as the urea nitrogen? Are other nitrogen constituents of the urine affected? Does the urea and non-protein nitrogen of the blood increase or decrease? And finally, for how long is it possible to depress urea excretion by the administration of these drugs? It was for the purpose of answering these questions that the following experiments were done.

EXPERIMENTAL. Male and female rabbits weighing approximately 2 kilos were placed in metabolism cages and given 500 grams of carrots and a cabbage leaf daily. On this diet they maintained a constant weight throughout the experimental period and produced an average of 250 cc. of alkaline urine daily. The urine was analyzed for total nitrogen by the micro-Kjeldahl method; urea by the method of Ormsby (4), ammonia by Folin's permutit method; allantoin by the method of Young and Conway (5) and creatinine by the method of Bonsnes and Taussky (6). Creatine could not be estimated since carrots contain some compound, which is also excreted in the urine, which reacts with picric acid on boiling. The blood was precipitated with phosphotungstic acid and the non-protein nitrogen and urea estimated by the micro-Kjeldahl and Ormsby methods. One cubic centimeter of blood from the ear vein sufficed for both determinations. The drugs were injected subcutaneously with sterile precautions in the morning and evening. One hundred milligrams of caffeine citrate, theobromine, or theophylline were injected each time in 3.0 cc. of water. Not all the theobromine would dissolve and therefore it was injected as a suspension. The actual caffeine dosage was half that of the other drugs because greater amounts of caffeine tended to make the animals hyperirritable.

After a period to establish the normal nitrogen excretion the drugs were injected for varying lengths of time. All three drugs produce the same effect but there is some variation from animal to animal. The nitrogen excretion may fall dramatically during the first day that the drug is given; it may fall slightly the first day, more the second and still more on the third; or there may be little effect on the nitrogen excretion for two or three days and then a rapid fall. These different patterns can be seen in the figures. But in all cases a significant fall in total and urea nitrogen excretion occurs and the ratio of the two remains constant. The other constituents of the urine show no consistent variations and when this was established no further analyses for them were done.

If this drop in nitrogen excretion was due to the inability of the kidney to excrete urea the urea-nitrogen of the blood should rise. As seen in figure 1 concomitant with the fall in excretion there is a lowering of blood urea and non-protein nitrogen concentrations. This indicates that there is an inhibition of

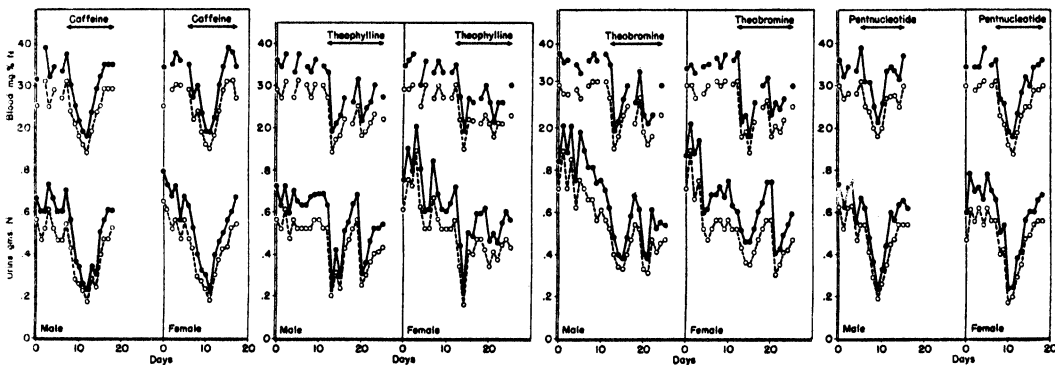


Fig. 1. The effect of injecting 100 mgm. of theobromine, theophylline and caffeine citrate and 350 mgm. pentnucleotide twice a day on the total and urea nitrogen excretion in the urine and the non-protein and urea nitrogen of the blood. The broken line represents urea nitrogen.

the formation of urea in the liver. In the rabbit the blood urea comprises 70–80 per cent of the non-protein nitrogen so that it is unlikely that other nitrogenous constituents are greatly affected.

Figure 1 shows that during prolonged administration of any of the drugs the blood and urine values tend towards normal, i.e., some tolerance develops. A possible mechanism for the tolerance is suggested by the *in vitro* experiments which showed that the inhibition of urea formation by the methyl xanthines can be overcome by the addition of either ornithine or glutamine. If the liver, *in vivo*, can make more of these compounds it should be able to overcome the inhibition. On the other hand if the drugs are given for a short period their effect can be repeated again when the nitrogen excretion has returned to normal. This is shown in figure 2.

Experiments were then done with pentnucleotides prepared from yeast by the Smith, Kline and French Laboratories. Three hundred and fifty milligrams

were injected twice daily. *In vitro* experiments with pentnucleotides were inconclusive because the liver deaminates them. But since these nucleotides are purines combined with pentose it was of interest to determine whether they could also inhibit urea formation. They stimulate white cell growth and may affect nitrogen metabolism. Figure 1 shows that this preparation of nucleotides in the dosage given does inhibit urea and total nitrogen excretion to approximately the same extent as the methyl xanthines.

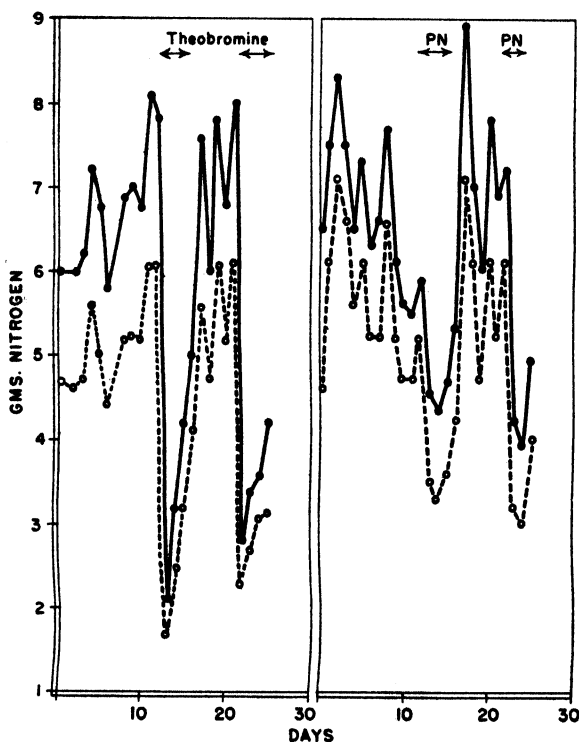


Fig. 2. The effect of two 3 day periods of injection of theobromine and pentnucleotide on the total and urea nitrogen excretion in the urine. The broken line represents urea nitrogen.

DISCUSSION. Fairly large doses of the methyl xanthines when injected into rabbits cause a decrease in nitrogen excretion because urea production is inhibited. The question arises concerning the fate of the nitrogen which would normally have been converted into urea, since neither it nor any other nitrogenous compound appears in greater amounts in the urine or in the non-protein nitrogen fraction of the blood. It is probable that under normal conditions ammonia derived from nitrogenous compounds is converted partly to urea and partly to compounds that are ultimately synthesized into protein. When urea formation is inhibited more ammonia is directed into anabolic pathways. The fact that pentnucleotides act similarly to the xanthines suggests that inhibition of urea

formation may be part of the mechanism by which pentnucleotides induce leucocytosis, or, in a more general sense, part of the mechanism by which growth of the organism as a whole is regulated by nucleoproteins and their derivatives.

SUMMARY

Caffeine, theobromine or theophylline injected into rabbits causes a decrease in the total and urea nitrogen of the urine, with no consistent variation in the concentration of the other nitrogenous compounds, and a decrease in the non-protein and urea nitrogen of the blood. Tolerance to this effect is gradually developed. Yeast pentnucleotides have a similar effect.

REFERENCES

- (1) BENEDICT, S. R. *J. Lab. Clin. Med.* **2**: 1, 1916.
- (2) BERNHEIM, F. AND M. L. C. BERNHEIM. *J. Biol. Chem.* **160**: 249, 1945.
- (3) BERNHEIM, F. AND M. L. C. BERNHEIM. *This Journal* **145**: 115, 1945.
- (4) ORMSBY, A. A. *J. Biol. Chem.* **146**: 595, 1942.
- (5) YOUNG, E. G. AND C. F. CONWAY. *J. Biol. Chem.* **142**: 839, 1942.
- (6) BONSNES, R. W. AND H. H. TAUSSKY. *J. Biol. Chem.* **158**: 581, 1945.

ADDENDUM

Two women, who were not habitual coffee or tea drinkers, were given 0.3 gram of theobromine with each meal for three days. Their control 24 hour total nitrogen excretion varied from 8.4 to 10.1 grams and from 10.2 to 12.4 grams respectively. After the third day of theobromine their total nitrogen excretion was 6.6 and 8.0 grams respectively. When they had returned to normal they were given the same dosage of theophyllin. After the third day their total nitrogen values were 5.4 and 6.0 grams respectively. The urea nitrogen decreased proportionately.

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CHANGES IN BLOOD ACID-BASE BALANCE DURING ASPHYXIA AND RESUSCITATION

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Previous studies on acid-base balance during asphyxia have dealt almost entirely with the earlier stages of the process before the occurrence of apnea (1). In addition, they have been primarily concerned with prolonged moderate anoxia rather than with acute asphyxia of the type which would be produced by tracheal obstruction or by the inhalation of an inert gas. The present study was undertaken to obtain more adequate information on acid-base balance during the late stages of acute asphyxia, particularly after the occurrence of apnea and during the application of artificial respiration. Data of this type should be of value in providing a better theoretical basis for certain resuscitative procedures.

METHODS. Dogs were used throughout this study. They were anesthetized with nembutal at a "physiologically standardized" dosage in order to avoid undue depression of the medullary centers (2). The control values for arterial pH and $p\text{CO}_2$ were within the normal range and indicate that there was little or no respiratory depression in these animals. The trachea was cannulated and attached to a calibrated McKesson anesthesia machine which provided any desired mixture of oxygen and nitrogen. By means of a three way valve, the animal could be disconnected from the anesthesia apparatus and attached instead to a positive pressure pump for artificial respiration. The length of stroke and rate of this pump could be varied to give any desired value for tidal air and minute volume. Respiratory minute volume was recorded by passing the expired air through a low resistance gas meter provided with contact points to record electrically on a smoked drum. Respiratory rate was recorded by means of a pneumograph and tambour. Blood pressure was recorded from the femoral artery. Ten cubic centimeter samples of circulating blood were obtained from a "T" cannula in the carotid artery, the animals having been previously heparinized in order to prevent clotting. The blood was run under oil into a special centrifuge tube containing fluoride and was centrifuged immediately in the completely filled stoppered tube as described by Peters and Van Slyke (3). The

plasma was then stored over mercury until the analyses were performed. All analyses were completed within 4 hours. The last few drops of each blood sample were run into a separate vessel and used for hemoglobin determinations.

Total plasma CO_2 content was determined by the manometric method of Van Slyke (3). Plasma pH was measured at 37.5°C . by means of a high resistance, syringe type glass electrode in conjunction with a Leeds and Northrup type K_2 potentiometer and a no. 7673 thermionic amplifier. This method has a maximum error of ± 0.01 pH unit. Hemoglobin was determined colorimetrically in an Evelyn photoelectric colorimeter.

From the measured values of plasma CO_2 content and pH, the pCO_2 and BHCO_3 content were calculated according to the Henderson-Hasselbach equation:

$$\text{pCO}_2 = \frac{(\text{CO}_2)}{0.0672 \left(\frac{7.9 \times 10^{-7}}{(\text{H}^+)} + 1 \right)} \quad (1)$$

and

$$\text{BHCO}_3 = (\text{CO}_2) - 0.0672 \text{pCO}_2 \quad (2)$$

In addition, plasma bicarbonate capacities for fully oxygenated blood at a standard pH of 7.40 were calculated according to the following equation (3):

$$(\text{BHCO}_3)_{7.40} = (\text{BHCO}_3)_{\text{pH}} + (\text{pH} - 7.40) (16.3 + 2.3 \text{Hb}_B) - 0.36 (\text{Hb}_B - \text{O}_{2(B)}) \quad (3)$$

where $(\text{BHCO}_3)_{7.40}$ is the plasma bicarbonate capacity at $\text{pH} = 7.40$, $(\text{BHCO}_3)_{\text{pH}}$ is the plasma bicarbonate content at the observed pH, Hb_B is the oxygen capacity in vol. per cent and $\text{O}_{2(B)}$ is the oxygen content in volumes per cent. This equation corrects the observed bicarbonate content for changes in pH, hemoglobin content, and oxygen saturation and is important in the proper interpretation of acid-base changes during asphyxia. The constant, 16.3, in equation 3, which represents the value of $\frac{\Delta \text{BHCO}_3}{\Delta \text{pH}}$ for plasma alone, is based upon human plasma

containing 70 grams of protein per liter with a normal albumin-globulin ratio of 1.6. Its exact value will vary with the total protein content and the albumin-globulin ratio (4). The value 16.3 should apply approximately to normal dog plasma in which the total protein content and the albumin-globulin ratio do not differ greatly from those of human plasma (5). It is to be remembered, however, that calculations based on equation 3 will give approximate values only in the absence of exact data for the values of the constants in each case.

RESULTS. I. Control Values for Dogs under Light Nembutal Anesthesia. Control values for all of the animals studied are summarized in table 1. The control blood samples were taken from 15–30 minutes after the completion of all surgical procedures and just prior to the initiation of asphyxia. The mean control values for the 28 dogs were: pH, 7.41; pCO_2 , 35.9 mm. Hg; and $(\text{BHCO}_3)_p$, 49.2 vol. per cent. The standard deviation of the distribution and the standard

error of the mean are indicated in the table for each value. These results may be compared with those of Shock and Hastings (6) on finger blood in unanesthetized human subjects. Their mean values for 57 individuals were: pH, 7.40; $p\text{CO}_2$, 42.86 mm. Hg; and $(\text{BHCO}_3)_p$, 62.15 vol. per cent. The fact that normal

TABLE 1
Control acid-base values for arterial plasma in dogs under light nembutal anesthesia

DOG NO.	SEX	pH	$p\text{CO}_2$ MM. HG	$(\text{BHCO}_3)_p$ VOLS. %
1	M	7.49	32.9	56.42
2	M	7.41	43.7	60.06
3	F	7.25	37.2	35.35
4	M	7.39	32.0	42.02
5	M	7.40	33.7	45.15
6	M	7.34	36.8	41.38
7	M	7.47	32.0	51.05
8	M	7.42	35.0	49.01
9	M	7.40	37.0	49.50
10	F	7.37	35.0	43.94
11	M	7.49	33.0	53.40
12	M	7.45	37.9	56.88
13	M	7.36	48.2	58.93
14	M	7.33	42.9	48.71
15	F	7.35	43.5	51.93
16	F	7.40	33.6	44.95
17	F	7.55	22.6	42.72
18	F	7.43	30.5	51.48
19	F	7.34	37.0	43.08
20	M	7.27	24.6	24.43
21	F	7.46	33.5	52.14
22	F	7.45	32.4	48.86
23	M	7.48	33.1	53.35
24	F	7.46	34.6	53.42
25	F	7.45	33.6	50.41
26	M	7.40	41.0	54.96
27	F	7.38	44.1	56.50
28	F	7.39	44.3	58.04
Mean.....		7.406	35.92	49.22
Standard deviation of distribution (σ).....		0.067	5.94	7.69
Standard error of mean (S).....		0.013	1.12	1.45

dogs have a lower plasma CO_2 combining power (method of Van Slyke and Cullen) than humans was reported by Haden and Orr (7), who found an average value of 34.8 vol. per cent with a rather wide range of variation.

The data of Shock and Hastings indicated a significantly lower value for $p\text{CO}_2$ and $(\text{BHCO}_3)_p$ in human females as compared to males. Our data, however, show no significant difference in these values for male and female dogs. Although the female dogs did show a slightly lower mean $p\text{CO}_2$ and $(\text{BHCO}_3)_p$

than the males, the number of animals used in this work is too small to warrant a final generalization on this point.

II. *Acid-Base Changes in Animals Dying of Asphyxia.* A. *Obstructive asphyxia.* A total of seven animals was studied in this series. Asphyxia was produced by tracheal obstruction which was maintained till the animal died. Arterial blood samples were taken at given time intervals throughout the course of asphyxia.

The average arterial pH fell steadily from a control value of 7.40 to reach 7.20 just before the circulation failed. The mean arterial $p\text{CO}_2$ rose steadily from a control value of 35.1 mm. Hg to reach a maximum of 64.9 mm. Hg. The calculated bicarbonate content rose at first and then began to fall. The bicarbonate capacities, calculated according to equation 3 above, indicate that a metabolic acidosis began early and increased rapidly during the later stages. Thus the bicarbonate capacity fell from a control value of 46.25 vol. per cent to 32.85 vol. per cent in the final stage of asphyxia. This represented a decrease of 28.9 per cent. Apnea occurred in these animals at an average interval of 2.6 minutes following tracheal obstruction and the blood pressure fell to zero after 6.4 minutes.

In 3 obstructed animals samples of cerebral venous blood were obtained from the Torcular Herophili. In these samples, the $p\text{CO}_2$ rose to an average maximum of 71.9 mm. Hg.

In figure 1 the average results described above are plotted on the logarithmic tri-axial graph introduced by Hastings and Steinhaus (8). The point "M.C." represents the mean control value for the 28 dogs presented in table 1 and the hexagonal area represents the range of normal variation. The curves marked "respiratory pathway" were calculated as described by Gray (9). The lower curve (Oxygenated) represents the CO_2 absorption curve for true plasma from fully oxygenated blood with an oxygen capacity of 21.2 vol. per cent and a bicarbonate capacity of 46.25 vol. per cent at a pH of 7.40. This curve goes through the average control point for this group of animals. The upper curve (reduced) represents the CO_2 absorption curve for true plasma from the same blood in the fully reduced state.

The curves marked "uncompensated metabolic pathway" were also calculated as described by Gray (10). They are based on the assumption that strong acid (or base) is added to blood under conditions such that no CO_2 can escape. Separate curves have been calculated for true plasma from fully oxygenated and from fully reduced blood having the oxygen capacity and bicarbonate capacity corresponding to the average control point for this group of animals. Displacement to the left along these pathways would indicate a metabolic acidosis completely uncompensated by respiratory activity.

It will be noted that the obstructed animals followed the respiratory pathway during the first 3 minutes during which there was a shift from the fully oxygenated to the fully reduced state. During the later stages, the pathway paralleled that of an uncompensated metabolic acidosis. These changes indicate an early respiratory acidosis with a metabolic acidosis superimposed, particularly in the later stages.

Comment. The interpretation of acid-base values during asphyxia are complicated somewhat by two other changes in the blood, an increase in hemoglobin concentration and a reduction in oxygen saturation. Both of these changes influence the height and slope of the CO_2 absorption curve and may thus modify the interpretation of acid-base data.

Table 2 presents data on hemoglobin concentrations in the seventeen animals in which this determination was made. In 15 of the 17 cases, asphyxia was

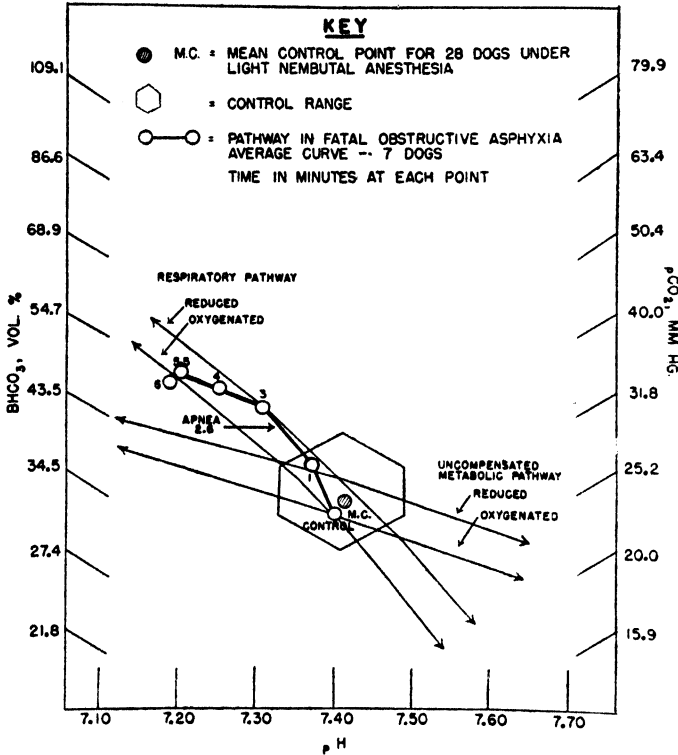


Fig. 1. Acid-base pathway in fatal obstructive asphyxia

accompanied by a considerable rise in hemoglobin concentration, the average increase for the entire series being 24.1 per cent. Hemoconcentration of similar degree has been observed during asphyxia by other investigators (11, 12), and has been attributed to contraction of the spleen (11) and possibly also to leakage of plasma through damaged capillaries (12). Autopsies performed on our animals after fatal asphyxia always revealed a dry, contracted spleen.

Data on arterial oxygen saturation during asphyxia have been reported by Birnbaum and Thompson (13). In obstructive asphyxia, the saturation fell to about 5 per cent in 3-4 minutes; in nitrogen asphyxia, it fell to 4 per cent in 2 minutes.

Both of the changes outlined above tend to mask the occurrence or severity of a metabolic acidosis developing during asphyxia. For example, a change from

complete oxygenation to complete reduction in blood with an oxygen capacity of 19.5 vol. per cent would increase the bicarbonate content of the true plasma by approximately 7 vol. per cent at any given pH (14). Further, an increase in hemoglobin concentration increases the buffer capacity of the blood against CO_2 .

Failure to correct for these two changes may cause considerable error in estimating the degree of metabolic acidosis. For example, in this particular group of animals, the calculated decrease in bicarbonate capacity in the last blood

TABLE 2
Changes in hemoglobin concentration during asphyxia

DOG NO.	CONTROL GRAMS/ 100 CC.	ASPHYXIA GRAMS/ 100 CC.	% CHANGE
1	16.5	24.2	+46.7
2	15.8	19.8	+25.3
3	15.8	16.7	+5.7
4	16.8	23.0	+36.9
5	15.6	19.6	+25.6
6	16.7	19.0	+13.8
7	17.2	19.1	+11.0
8	14.0	17.4	+24.3
9	13.5	16.3	+20.7
10	13.7	18.5	+35.0
11	21.8	21.8	0.0
12	16.3	18.0	+10.4
13	15.5	18.4	+18.7
14	14.9	18.7	+25.5
15	16.5	18.9	+14.5
16	12.3	15.7	+27.6
17	15.5	15.9	+2.6
Mean.....	15.8	19.6	+24.1
Standard deviation of distribution (σ).....	2.02	2.47	
Standard error of mean (S).....	0.496	0.599	

Standard error of difference of means (S_d)..... 0.777

Mean Difference
 S_d 4.89

sample was 28.9 per cent. If changes in oxygen saturation and hemoglobin content had been ignored, the calculated change would have been only 9.5 per cent.

Similarly, these changes tend to distort the acid-base pathways as plotted on the tri-axial graph and this must be kept in mind in attempting to interpret these curves. Thus, the early upward displacement of the pathway plotted in figure 1 is due primarily to a reduction in oxygen saturation and this may mask the beginning of a metabolic acidosis.

B. *Nitrogen asphyxia*. Seven animals were asphyxiated by the inhalation of 100 per cent nitrogen and allowed to proceed to a fatal termination. During the

first minute, the average arterial pH rose rapidly to reach 7.72 at the height of the hyperventilation. At the same time, the mean arterial $p\text{CO}_2$ fell rapidly to a minimum of 16.8 mm. Hg. Then, as respiration slowed and apnea occurred, the $p\text{CO}_2$ rose rapidly while the pH fell. By the time circulation failed, $p\text{CO}_2$ had risen to 39.8 mm. Hg which was higher than the control value of 36.1 mm., and the pH had fallen to 7.37 which was below the control value of 7.43.

The calculated bicarbonate capacity, after an apparent increase during the first minute, decreased rapidly to reach a value of 39.08 vol. per cent after 5.5

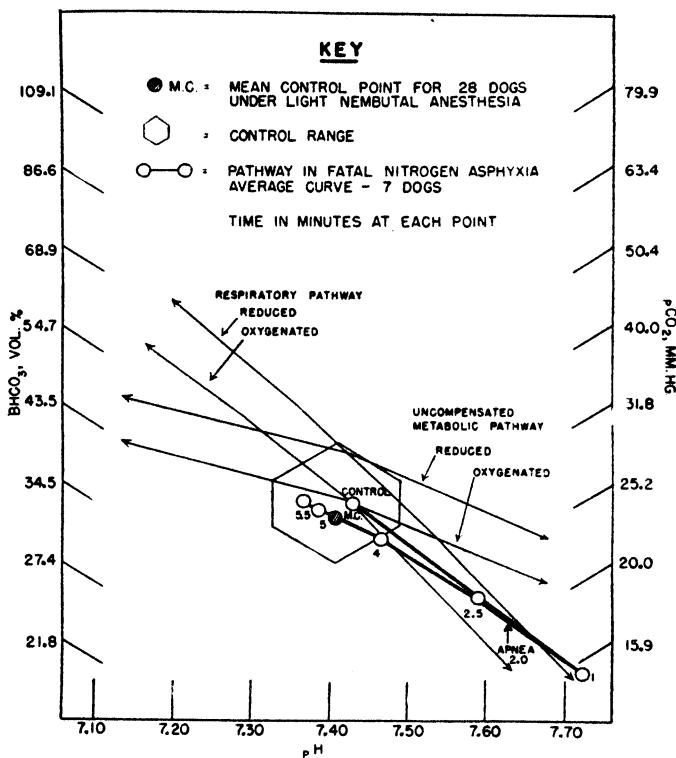


Fig. 2. Acid-base pathway in fatal nitrogen asphyxia

minutes. This represented a fall of 33.6 per cent. The reason for the apparent increase in bicarbonate capacity during the first minute is not clear.

The acid-base pathway is plotted in figure 2. A marked respiratory alkalosis occurred initially during the hyperventilation. The pathway is somewhat distorted in this first phase because of the progressive reduction in oxygen saturation. With respiratory depression and apnea, the pathway travelled nearly parallel to the uncompensated metabolic pathway with a rise in $p\text{CO}_2$ and fall in pH. On the average, apnea occurred in these animals 2 minutes after nitrogen inhalation had begun, and the blood pressure fell to zero after 5.5 minutes.

Comment. These results indicate that during fatal obstructive asphyxia, an early respiratory acidosis occurs followed by an uncompensated metabolic acidosis. As a result, arterial $p\text{CO}_2$ and $[\text{H}^+]$ rise to extremely high levels. During fatal nitrogen asphyxia, a profound respiratory alkalosis occurs during the early stage of hyperventilation. After apnea occurs, a rapidly developing metabolic acidosis uncompensated by respiratory activity results in a rapid rise in arterial $p\text{CO}_2$ and $[\text{H}^+]$ both of which usually reach supernormal values by the time circulation fails.

III. *Acid-Base Changes in Animals Resuscitated from Asphyxia.* A. *Obstructive asphyxia.* Having established the acid-base pathways in two types of fatal asphyxia, the question arose as to how they are modified by the application of artificial respiration. It has been suggested, for example, that if artificial ventilation of the lungs were instituted during the stage of apnea, arterial $p\text{CO}_2$ levels might drop rapidly to low levels. To answer this question a second series of experiments was performed.

In this series, the animals were asphyxiated by tracheal obstruction and artificial respiration with air at the animal's control respiratory rate and tidal volume was instituted at intervals ranging from 1.00 to 1.75 minutes after the beginning of apnea. Five animals, all successfully resuscitated, were studied. Blood samples were taken throughout the course of asphyxia, artificial respiration and recovery.

All five animals gave results which were qualitatively similar and which are illustrated by the typical experiment (dog 5) summarized in figure 3. Because of the variations in the length of apnea, and the time of application and duration of the artificial respiration, no attempt was made to obtain an average for the five animals studied. Variations from the "typical experiment" in the individual animals will be described later.

As illustrated in figure 3, displacement occurred along the respiratory pathway following tracheal obstruction indicating a respiratory acidosis. One and two-tenths minutes after the beginning of apnea and just before artificial respiration was begun, arterial $p\text{CO}_2$ had risen from a control value of 44.3 mm. Hg to 67.4 mm., and the pH had fallen from 7.39 to 7.28. At this time the blood pressure had fallen to 30 mm. Hg. A blood sample, taken 2.4 minutes after artificial respiration was started, showed that both $p\text{CO}_2$ and $[\text{H}^+]$ continued to rise during artificial respiration. The blood pressure at this time had risen to 160 mm. Hg. The pathway is that of a partially compensated metabolic acidosis.

The animal's first spontaneous gasp occurred after 2 minutes of artificial respiration and the pump was stopped after 2.8 minutes. After 2.4 minutes of spontaneous breathing, a blood sample revealed that the pH had continued to fall and the $p\text{CO}_2$ to rise. The animal's respiration during this period was very slow and his minute volume was only about 500 cc. compared to his control value of 1200 cc. That this was not due to any deficiency in chemical stimuli is indicated by an arterial $p\text{CO}_2$ of 76.8 mm. and a pH of 7.15 at this time.

As recovery occurred, the animal began to hyperventilate in an apparent attempt to lower the arterial $p\text{CO}_2$ level and compensate for the metabolic acido-

sis. Still later, there was some increase in the bicarbonate capacity. This was most probably due to oxidative removal of part of the accumulated lactic acid with release of fixed base.

The calculated bicarbonate capacities revealed a progressive metabolic acidosis. There was a maximum decrease of 39.2 per cent in the bicarbonate capacity after 20 minutes. Following this, the values began to increase again.

Individual variations occurred from this typical picture in some of the animals. For example, in one animal (no. 1), the $p\text{CO}_2$ remained almost constant

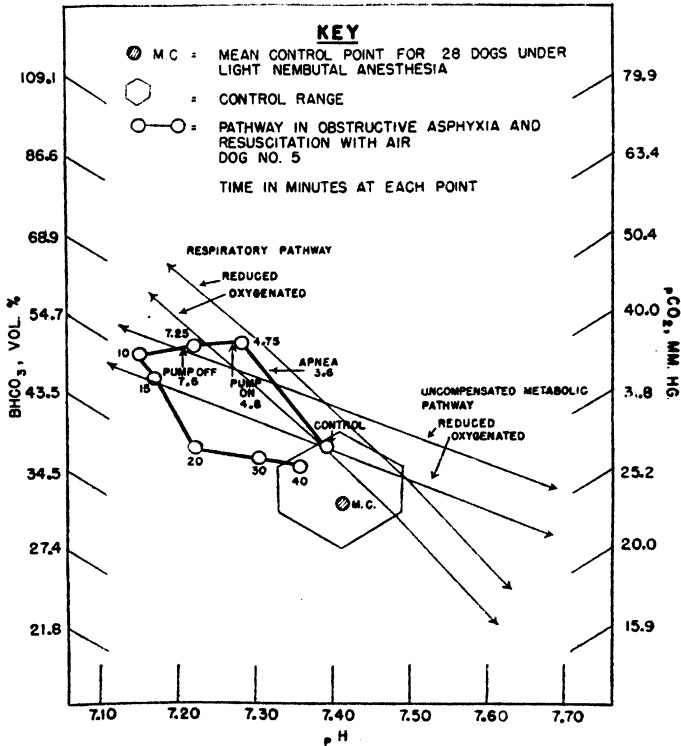


Fig. 3. Acid-base pathway during obstructive asphyxia and resuscitation with air

at 55 mm. during the first minute of artificial respiration and then fell during the second minute and one-half to 50 mm. In another animal (no. 4), the $p\text{CO}_2$ dropped during the first minute of artificial respiration from 50 to 43 mm. However, at no time during artificial respiration in any of the animals did the arterial $p\text{CO}_2$ and $[\text{H}^+]$ fall to normal or subnormal levels. In all cases, both $p\text{CO}_2$ and $[\text{H}^+]$ were considerably above the control values at the time artificial respiration was discontinued.

B. Nitrogen asphyxia. Animals in this series were asphyxiated by nitrogen inhalation and artificial respiration with air at the animal's normal rate and tidal volume was initiated at intervals ranging from 1.3 to 2.9 minutes after the beginning of apnea. Five animals, all successfully resuscitated, were studied.

All five animals gave qualitatively similar results, illustrated by the typical experiment summarized in figure 4 (dog 3).

As shown in figure 4, a profound alkalosis occurred during the first stage of asphyxia when hyperventilation was extreme. Arterial $p\text{CO}_2$ fell from 33.6 to 18.5 mm. Hg and the pH rose from 7.45 to 7.69. During apnea the pathway paralleled that of uncompensated metabolic acidosis. After 2 minutes of apnea

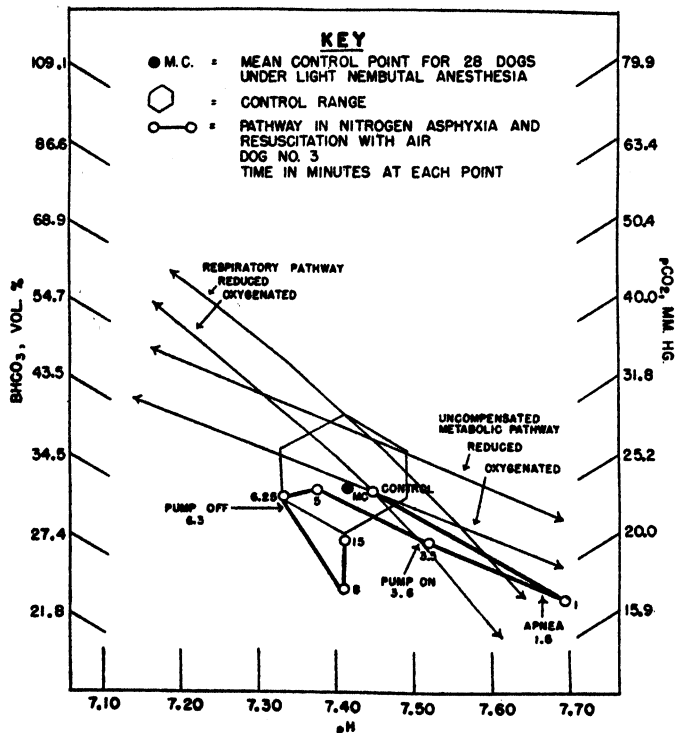


Fig. 4. Acid-base pathway during nitrogen asphyxia and resuscitation with air

and just before artificial respiration was instituted, $p\text{CO}_2$ had risen to 27.2 and pH had fallen to 7.52. During the first 1.4 minutes of artificial respiration the pathway continued parallel with the uncompensated metabolic curve. The $p\text{CO}_2$ continued to rise to 37.9 mm. and the pH fell to 7.37. During the next 1.2 minutes, the $p\text{CO}_2$ rose slightly to 38.2 mm. and the pH fell to 7.33. When artificial respiration was discontinued, the animal hyperventilated in an attempt to compensate his metabolic acidosis. After 1.7 minutes of spontaneous breathing, arterial $p\text{CO}_2$ was reduced to 26.4 mm. Hg but the pH was still slightly below its control value.

The calculated bicarbonate capacities revealed a progressive fall after an apparent increase during the first minute. A maximum fall of 31.9 per cent occurred after 8 minutes. Following this, the values began to increase again.

Although individual animals differed quantitatively from this typical picture, it should be noted that in every case the arterial $p\text{CO}_2$ and $[\text{H}^+]$ continued to rise during artificial ventilation and that in 4 of 5 cases, they were above the control values at the time artificial respiration was discontinued.

Comment. These results indicate that artificial respiration with air at a normal rate and tidal volume applied to animals apneic as a result of nitrogen asphyxia does not lower arterial $p\text{CO}_2$ and $[\text{H}^+]$. On the contrary, both values continue to rise during artificial ventilation and usually reach supernormal levels by the time spontaneous respiration is resumed.

DISCUSSION. Earlier work which questioned the occurrence of acidosis during asphyxia was directed primarily toward the earlier stages of moderate anoxia and did not consider the late, post-apneic period of acute asphyxia (1).

Studies of acid-base changes during the later stages of asphyxia have been reported by Kohler et al. (15). These authors found that a severe acidosis occurred in the terminal stages, but only a few animals were studied after the occurrence of apnea. In asphyxia neonatorum Eastman (16) has demonstrated the existence of a severe acidosis with extremely high levels of arterial $p\text{CO}_2$ and $[\text{H}^+]$.

Our own results indicate that in the two types of acute asphyxia studied, a rapidly developing metabolic acidosis occurs in the late stages. After apnea occurs, this metabolic acidosis is completely uncompensated by respiratory activity and, as a result, the arterial $p\text{CO}_2$ and $[\text{H}^+]$ rise rapidly (10). In obstructive asphyxia, the arterial levels of these chemical agents rise to extremely high values as there is no initial alkalosis to overcome. In nitrogen asphyxia, the late metabolic acidosis is sufficient completely to overcome the initial alkalosis and allow arterial $p\text{CO}_2$ and $[\text{H}^+]$ to reach normal or high values by the time circulation fails.

Our results indicate further that during artificial respiration with air at a normal minute volume, arterial $p\text{CO}_2$ does not fall but usually continues to rise. In all cases both $p\text{CO}_2$ and $[\text{H}^+]$ were above normal when spontaneous respiration was resumed. The question arises as to why arterial $p\text{CO}_2$ and $[\text{H}^+]$ continue to rise during artificial respiration. Three factors appear to be important in explaining this observation: 1. Oxygenation of the hemoglobin increases the base binding capacity of the latter thus taking base from bicarbonate, releasing free CO_2 , and lowering pH. 2. Metabolic acids formed during asphyxia accumulate in the tissues and capillary beds as the circulation fails. When the circulation recovers during resuscitation, these acid products are swept into the general circulation and release CO_2 from bicarbonate. 3. Aerobic production of CO_2 is resumed. Apparently, artificial respiration at a normal minute volume is not adequate to eliminate this load of CO_2 so that arterial $p\text{CO}_2$ continues to rise and pH to fall.

It would appear from these results that in the two types of asphyxia studied, it is unnecessary to add CO_2 to the resuscitative mixture in order to maintain normal arterial $p\text{CO}_2$ levels during the application of artificial respiration. This would be true as long as the lung ventilation did not exceed the average normal

minute volume, and the latter is unlikely with most methods of artificial respiration currently in use (17).

SUMMARY AND CONCLUSIONS

1. Acid-base changes in the arterial blood of dogs during fatal asphyxia and during successful resuscitation have been studied. Asphyxia was produced by tracheal obstruction or by nitrogen inhalation.

2. During fatal obstructive asphyxia, there is an early respiratory acidosis followed by an uncompensated metabolic acidosis. By the time circulation fails, arterial $p\text{CO}_2$ reaches an average value of 64.9 mm. Hg, and the pH falls to 7.20.

3. In fatal nitrogen asphyxia, the early respiratory alkalosis is overcome by a late respiratory and metabolic acidosis so that both arterial $p\text{CO}_2$ and $[\text{H}^+]$ are slightly above normal when circulation fails.

4. During artificial respiration with air at the animal's control rate and tidal volume, arterial $p\text{CO}_2$ and $[\text{H}^+]$ continue to rise in most cases. In all cases, both the $p\text{CO}_2$ and $[\text{H}^+]$ remain above normal until spontaneous respiration is resumed. On restoration of spontaneous breathing, hyperventilation occurs in an attempt to lower the $p\text{CO}_2$ and compensate the metabolic acidosis.

5. These results indicate that there is no deficiency in the arterial levels of $p\text{CO}_2$ or $[\text{H}^+]$ during the entire course of obstructive asphyxia or in the later stages of nitrogen asphyxia. They further indicate that artificial respiration with air at a normal minute volume does not produce a deficiency in the levels of these agents in the arterial blood. Thus it would appear to be unnecessary to include CO_2 in the resuscitative mixture in order to maintain normal arterial levels of $p\text{CO}_2$ during artificial respiration.

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REFERENCES

- (1) HENDERSON, Y. AND H. W. HAGGARD. Noxious gases. Reinhold, New York, 1943.
- (2) WIGGERS, C. J. *Physiol. Reviews* **22**: 74, 1942.
- (3) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Vol. II Methods. Williams & Wilkins Co., Baltimore, 1937.
- (4) VAN SLYKE, D. D., A. B. HASTINGS, A. HILLER AND J. SENDROY. *J. Biol. Chem.* **74**: 769, 1928.
- (5) HOWE, P. E. *Physiol. Reviews* **5**: 439, 1925.
- (6) SHOCK, N. W. AND A. B. HASTINGS. *J. Biol. Chem.* **104**: 585, 1934.
- (7) HADEN, R. L. AND T. G. ORR. *J. Biol. Chem.* **65**: 479, 1925.
- (8) HASTINGS, A. B. AND A. H. STEINHAUS. *This Journal* **96**: 538, 1931.
- (9) GRAY, J. S. AAF School of Aviation Medicine. Research Project no. 386, Report No. 1, 1 May 1945.
- (10) GRAY, J. S. AAF School of Aviation Medicine. Research Project no. 386, Report no. 2, 20 November 1945.

- (11) BARCROFT, J. Features in the architecture of physiological function. Cambridge University Press, 1938.
- (12) MULLIN, F. J., J. DENNIS AND D. B. CALVIN. *This Journal* **124**: 192, 1938.
- (13) BIRNBAUM, G. L. AND S. A. THOMPSON. *J. A. M. A.* **118**: 1364, 1942.
- (14) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Vol. I. Interpretations. Williams & Wilkins Co., Baltimore, 1937.
- (15) KOEHLER, A. E., H. M. F. BEHNEMAN, O. E. BENELL AND A. S. LOEVENHART. *This Journal* **74**: 590, 1925.
- (16) EASTMAN, N. J., R. B. DUNN AND J. KREISELMAN. *Am. J. Obstet. and Gynecol.* **36**: 571, 1938.
- (17) COMROE, J. H., JR. AND R. D. DRIPPS, JR. *Ann. Rev. Physiol.* **7**: 653, 1945.

THE EFFECTS OF HEMORRHAGE ON TISSUE METABOLITES¹

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Analyses of tissues for key metabolites were used in this laboratory to study the biological energy transformations in several types of experimental shock (1). It became appropriate to extend these studies, previously carried out with rats in shock produced by trauma, leg tourniquets or toxic doses of barbiturates, to hemorrhaged rats. From our earlier findings, it seemed that freezing animals in liquid air for analysis just as respiration ceased, while the hearts were still beating, would provide the most useful information. This enables us to determine, by analysis of the major tissues for their energy reservoirs, which of these tissues was most affected, and whether any of them were dead in the biochemical sense. A tissue is assumed to be dead, in the biochemical sense, when the immediately available energy reservoirs—the adenosine polyphosphates—are completely lacking.

The use of hemorrhage as the experimental method raises the issue of differentiating between acute blood loss and true hemorrhagic shock. Since it is difficult to draw any sharp line between the two conditions, and since both merit study, a series of animals was hemorrhaged in such a manner as to include both the above-mentioned situations, so that any major differences might be observed.

Preparation of animals. Sprague-Dawley male albino rats were obtained and kept 2 to 4 days in our laboratory before use. When taken for experiment, they weighed 220 to 250 grams. Such rats, under ordinary laboratory conditions have blood constituting approximately 6.7 per cent of their body weight. They were given a short exposure to ether, just sufficient to quiet them for a few seconds, and put into slings similar to that described by Haist and Hamilton(2). No further anesthesia was given, since it was desired to study the effects of hemorrhage uncomplicated by the side effects of anesthesia. The tails were put through clamped short glass tubes to hold them in position. The bleeding was carried out with a "citrate" method suggested by Drs. R. K. Meyer and Elva G. Shipley. The end of the tail was in each case cut off cleanly with a sharp razor and inserted into a graduated 15 ml. centrifuge tube containing 10.0 ml. of 2 per cent sodium citrate (anhyd.). The citrate prevents clotting of the blood at the cut surfaces and facilitates measurement of the volume of blood withdrawn. Depending on the rate of bleeding desired, 15 to 30 mm. was cut from the tail.

The volume of blood lost was read on a graduated tube at 5 minute intervals. The tubes were changed when necessary and the blood loss plotted at once on a graph as the percentage of the body weight. The rats were arranged on a rack

¹The work described in this paper was carried out under a contract recommended by The Committee on Medical Research, between The Office of Scientific Research and Development and The University of Wisconsin.

where several were bled at once and their bleeding and respiratory rates followed closely. The temperature of the room was maintained at 78 to 80° F. throughout the experiments.

Once experience was gained, it was possible to predict with considerable success the survival time after hemorrhage. Although one cannot draw a sharp line between the two types of animals, it seems probable that animals dying very soon after completion of the hemorrhage were cases of acute blood loss, and that those living for several hours following termination of the bleeding were cases of hemorrhagic shock. The latter had time to develop the characteristic chain of events associated with all forms of shock. The former suffer from acute general tissue hypoxia and perhaps do not have time to develop the same sequence of events.

Factors concerned in deciding when to ligate the rat's tail and stop hemorrhage in order to obtain an animal which would live a given period are as follows: 1, the amount of blood lost; 2, the rate at which it was lost; 3, the respiratory rate. When bleeding is rapid, it is not necessary to have as large a loss to kill the animal. An animal which is excited and struggles always bleeds much more rapidly. Because the bleeding rate is a reflection of blood pressure, it is critical to observe whether the bleeding curve has changed slope. In a rapidly bled animal, if bleeding is continued after the rate has levelled off, the animal will die very soon. In an animal bled slowly it is necessary to continue hemorrhage until the rate has levelled off considerably if the animal is to develop fatal shock. The respiratory rates are readily observed while the animals are in the slings. The rate before hemorrhage is approximately 80 per minute, which is maintained until the hemorrhage gets well under way. When 2 per cent of the body weight has been bled out there is usually an increase to 90 to 100. When the rate rises much above 120 per minute, the tail must be ligated unless one wishes the animal to die soon. When it reaches 150 to 180 the animal has almost certainly reached the lethal stage of hemorrhage. A rate of 180 usually portends death within 20 minutes.

Study of metabolites. From a considerable number of rats hemorrhaged, 11 were selected to fall in the desired experimental pattern, were frozen in liquid air at death and analyzed by methods previously described (1). The question of the use of anesthesia in freezing has been discussed in the previous publication, which demonstrated that when normal rats were dropped into liquid air there was sufficient lag in freezing to permit an almost quantitative splitting of adenosine triphosphate to adenosine diphosphate. This sudden breakdown was due to nerve stimuli which could be blocked by the administration of nembutal immediately before freezing. No anesthesia could be used with the hemorrhaged animals and none was necessary. They are completely insensitive to tail pinching for a period before death and should not require an anesthetic to prevent the stimulation by liquid air. However, controls frozen with and without anesthesia are provided for comparison.

Analyses were carried out on samples of brain, liver, kidney, skeletal muscle and heart from each animal. Tissue components measured were adenosine

triphosphate, adenosine diphosphate, adenylic acid, pentose phosphate, phosphocreatine, total acid-extractable phosphorus, inorganic phosphorus, glycogen, lactic acid, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, hexose diphosphate, phosphoglyceric acid, phosphopyruvic acid and "coenzyme." The latter is an adenine-nicotin-amide component which has no significance ex-

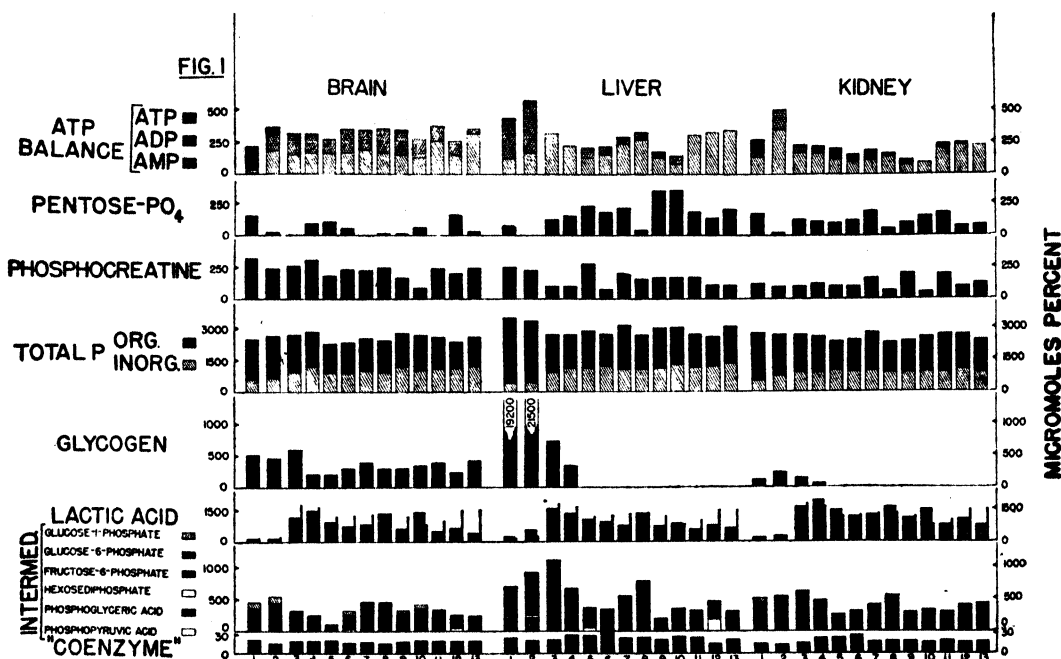


Fig. 1. Results of analyses for adenosinetriphosphate and metabolically related substances in brain, liver, and kidney following fatal hemorrhages in unanesthetized rats. In the case of lactic acid, the narrow bar to the right of the large bar indicates the amount of lactic acid in the blood of the same animal at death. The numbers along the bottom of the chart refer to individual animals in the case of the bled rats, which are numbered from 3 to 13. The amount and rate of bleeding and time of survival are given in table 1. The animals are arranged according to the survival time, with the longest survival at the right. Columns 1 and 2 represent control data. Column 1 is the average of 6 control animals given nembutal just before freezing, while column 2 represents the average of 5 control animals frozen without anesthesia.

cept as a correction for adenylic acid measurements. The results of the analysis are given in figures 1 and 2. Numbers 3 to 13 inclusive represent 11 hemorrhaged rats. The same number is used to denote the tissue components of the animal throughout figures 1 and 2 and in the data for bleeding rates given in table 1. No. 1 in figures 1 and 2 represents the averages of analyses on 6 control rats anesthetized by intraperitoneal injection with nembutal, 50 mgm./kgm. body weight, and frozen in liquid air as soon as surgical anesthesia was reached (3-4 min.). No. 2 represents the averages of analyses on the tissues of 5 control rats

frozen without anesthesia. Omissions in the plotting of the figure indicate that the component was not present in measurable quantity.

Additional information concerning metabolic events in these same experimental animals was provided by analyses of the blood for lactic acid. For these

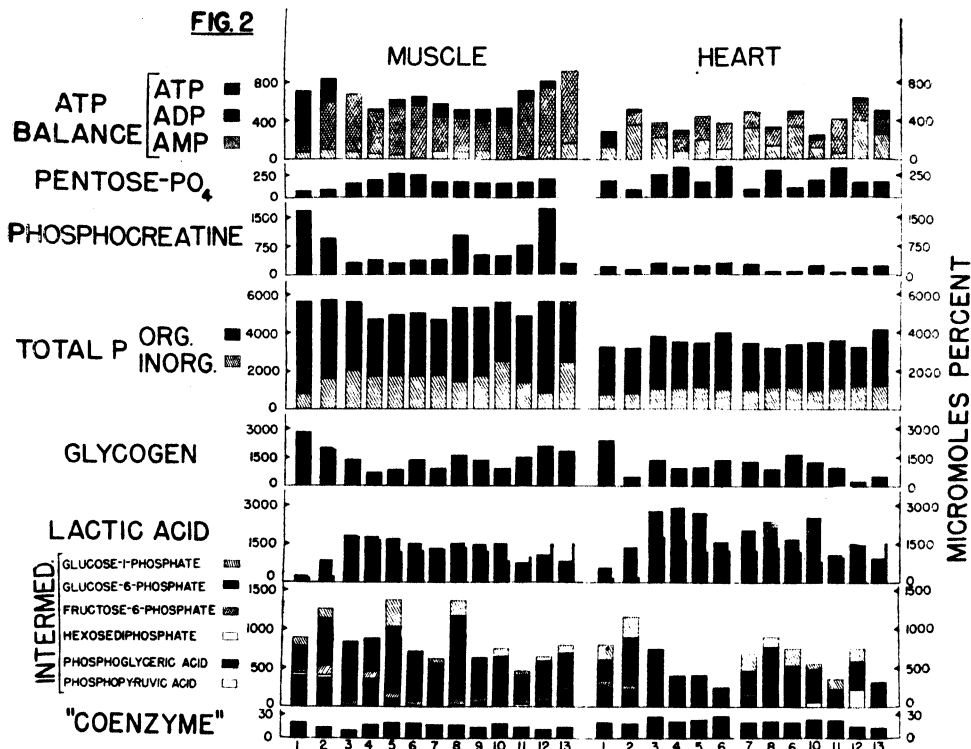


Fig. 2. Results of analyses for adenosinetriphosphate and metabolically related substances in skeletal muscle and heart muscle following fatal hemorrhages in unanesthetized rats. In the case of lactic acid, the narrow bar to the right of the large bar indicates the amount of lactic acid in the blood of the same animal at death. The numbers along the bottom of the chart refer to individual animals in the case of the bled rats, which are numbered from 3 to 13. The amount and rate of bleeding and time of survival are given in table 1. The animals are arranged according to the survival time, with the longest survival at the right. Columns 1 and 2 represent control data. Column 1 is the average of 6 control animals given nembutal just before freezing, while column 2 represents the average of 5 control animals frozen without anesthesia.

analyses 0.05 ml. samples were taken from the tails at the times indicated in table 1. Samples for blood lactic acid analyses at death were obtained by dissection of frozen blood from the heart auricles and from the vena cava. Both sources gave identical results and are probably valid for pooled venous blood. The results are recorded in table 1. The analyses are arranged, in general, in order of decreasing rate of blood loss and increasing survival time of the animals. No.

14, although it was not taken for complete analysis, was included to illustrate changes in blood lactate. Nos. 3 and 4 are assumed to be cases of acute blood loss. Nos. 11, 12, 13 and 14 are assumed to be cases of hemorrhagic shock. It

TABLE 1
Rate of bleeding and lactic acid content of blood in hemorrhaged rats

RAT NO.	TIME TAKEN IN BLEEDING	TOTAL TIME FROM START OF BLEEDING TO DEATH	AMOUNT OF BLOOD LOST. PER CENT OF BODY WEIGHT	BLOOD LACTIC ACID		
				Time sample taken	Amount	At death
	min.	min.		min.	mgm. %	mgm. %
3	55	75	2.52	55	119	160
4	70	94	2.88	80	140	148
5	68	105	3.45	75	118	107
6	95	142	3.45	95	105	112
7	95	151	2.86	95	101	117
8	70	105	3.28	70	118	121
9	125	187	3.32	90	73.7	109
10	190	195	3.50			73.8
11	135	273	3.49	155	52.7	88.5
12	175	765	3.42	175	45.6	134
13	50	790	3.33	120	44.0	137
14	105	632	3.25	440	41.7	109
				520	34.7	

TABLE 2
Post mortem changes in energy reservoirs and lactic acid
All results expressed in micromoles per cent

TREATMENT	INTERVAL BETWEEN DEATH AND FREEZING	BRAIN			LIVER			HEART		
		γ-P	Glyco- gen	Lactic acid	γ-P	Glyco- gen	Lactic acid	γ-P	Glyco- gen	Lactic acid
Nembutal plus de- capitation	2.5''	186	285	237	446	18,950	345	277	1,283	816
Decapitation	2.5''	188	344	192	331	19,150	372	93	395	930
Nembutal plus de- capitation	20'	71.5	100	868	247	6,290	940	40	0	1,470
Asphyxiation (N ₂)	20'	10.8	0	1,002	0	2,230	1,190	0	0	814
Decapitation	20'	0	0	822	152	3,840	1,360	23	0	2,970
Decapitation	40'	0	0	934	0	734	1,280	0	0	2,950

is less certain to which classification nos. 5 to 10 belong. They are somewhat intermediate between the two classifications.

In a normal rat at rest, the lactic acid of the blood is probably the product of glycolysis in the erythrocytes (3), and there is little addition of lactic acid to the blood by the tissues. However, during stress brought about by hemorrhage, the lactic acid level in the tissues rises markedly and certain of the tissues con-

tribute lactic acid to the blood, while others remove a part of it. The heart muscle is probably the most active in oxidation of lactate (4) while liver normally converts lactate to glycogen. Muscle tends to contribute lactate to the blood. In order to illustrate just which tissues were contributing to the elevated blood lactate at the time these experimental animals died the blood lactic acid levels found have been plotted in figures 1 and 2 beside the lactic acid analysis for each tissue of each animal, the blood lactic acid value appearing as a shorter column on the right where it is lower than the tissue level, as a narrow higher bar where it is higher and as a solid bar where identical with the tissue lactic acid level.

Since conclusions to be drawn from the data obtained by analysis of hemorrhaged rats at death are in part dependent upon comparison with post mortem changes, some examples of analyses on animals, following death by various means, are given in table 2. The data presented include easily hydrolyzable phosphorus (ATP + ADP), glycogen and lactic acid.

DISCUSSION. To appraise data from rats which died following hemorrhage it is necessary to compare them not only with the controls shown in columns 1 and 2 of figures 1 and 2, but also with data from animals which died sudden and violent deaths, and with the changes occurring in the tissues of such animals following death. The latter type of data are provided in table 2.

One notable feature of the analytical studies is the relatively good condition of brain samples from both acute blood loss and hemorrhagic shock cases. Biochemically speaking, these specimens were not dead, for they contained appreciable amounts of ADP, glycogen and phosphocreatine. Yet all these animals had suffered respiratory failure. Respiratory failure would be expected to result only when high energy phosphate reservoirs were depleted in the respiratory center, and we are forced to conclude that the analyses of whole brains bear no relation to the quantities of the labile energy reservoirs in the respiratory center. This is technically possible because of the disparity in mass between respiratory center and whole brain. The known anoxic survival times of cerebral tissue and brain stem are 5 minutes and 30 minutes respectively (5). However, energy reservoirs available at any time are dependent on the dynamic equilibrium between supply and demand. The mass of cerebral tissue has been depressed in functional level, while the respiratory center has been subjected to a prolonged hyperactivity which may have exhausted its energy reservoirs without so affecting those of the cerebral tissues.

A second possibility, that there are energy reservoirs more critical than those here determined, is contraindicated by the rapid disappearance of these energy reservoirs of cerebral tissue shown in the animals killed by decapitation and complete asphyxia in nitrogen.

A third possibility is that the respiratory center does still possess energy reservoirs, but that a breakdown in synaptic conduction has resulted in the loss of nervous mechanisms which normally activate it. Thus respiratory failure could be due to a failure of the reflex mechanisms, e.g., from the carotid sinus.

The condition of the heart samples, indicated by presence of some ADP, phosphocreatine and glycogen, was relatively good, although, as in brain, the

inorganic phosphate and lactate are greatly elevated. Here function was definitely present at the time of freezing. Because freezing occurred so soon after respiratory failure, while the heart was still functioning and not yet anoxic, the heart samples from hemorrhaged rats are in marked contrast to those in table 2.

The energy reservoirs of the muscle samples were relatively well preserved. In the earlier paper (1) it was shown that 4 hours' complete ischemia are necessary completely to deplete the high energy stores of muscle. In the muscles of these hemorrhaged rats the blood flow, though reduced, was apparently sufficient to prevent complete anaerobiosis. In addition the muscles were flaccid and probably less subject to stimulation than those of normal animals.

The liver and kidney samples provide a marked contrast to the other tissues. In five liver samples and two kidney samples no ATP or ADP could be detected. Furthermore only the two cases of very acute blood loss showed any glycogen in either liver or kidney, while in all the hemorrhagic shock cases none could be detected. Thus the livers of hemorrhagic shock cases contained less glycogen than animals dead for 40 minutes. The poor condition of these tissues biochemically is in accord with reports on depressed function in liver and kidney during shock (6, 7). The blood supply to these organs was quite evidently not maintained to the extent it was in brain and heart.

While failure of one of these organs, liver or kidney, may conceivably be the primary cause of death, such failure must have had its effect by impairment of energy mobilization in the respiratory center.

A feature of the data in figures 1 and 2 which may contribute to understanding of the vicious cycle involved in development of shock after hemorrhage is the marked rise of inorganic phosphorus and lactic acid in all the tissues. Both of these represent net increases in the ionic strength of the protoplasm in which they are found, and would consequently result in a movement of water into the cells from the intercellular fluid and the blood. To get an estimate of the quantitative significance of these shifts one has only to note that physiological saline represents 15,300 micromoles per cent when converted to the terms in which all data in figures 1 and 2 are reported. In animal 3, for example, elevations in the lactic acid and inorganic phosphate result in an increased ionic strength of approximately 17 per cent in terms of the isotonic saline. Due to the mass of muscle, and prevalence of this situation in the other tissues, the increased osmotic pressure would have a tendency to attract a considerable fraction of the body water, and would accentuate further the loss in circulating blood volume which is initiating the vicious cycle of shock. This loss of water from the blood would not be dependent upon alterations in capillary permeability.

In the late hemorrhagic shock cases, the blood has a higher lactic acid level than the tissues. This may be a technical effect due to a terminal rise in lactic acid. The blood samples taken from these animals prior to the terminal respiratory failure show much more moderate elevation in lactic acid analyses.

The tendency toward higher lactic acid levels in the rapidly fatal cases than in later deaths parallels our earlier findings in Noble-Collip and tourniquet shock (1). As in the other types of shock studied, there was apparently a defective

phosphate transfer, and phosphopyruvic acid accumulations were found, along with residues of phosphocreatine, in tissues depleted of adenosine polyphosphates.

SUMMARY

A method is described for hemorrhaging rats to experimentally produce cases of acute blood loss and hemorrhagic shock. Rats so hemorrhaged were frozen in liquid air and analyzed for a variety of tissue components, including the high energy phosphate reservoirs and intermediates of carbohydrate metabolism. Complete depletion of these energy reservoirs was found in some tissues from hemorrhaged animals. The differences between animals expiring in hemorrhagic shock and those meeting a rapid death with post mortem changes were illustrated.

REFERENCES

- (1) LePAGE, G. A. This Journal **146**: 267, 1946.
- (2) HAIST, R. E. AND J. J. HAMILTON. J. Physiol. **102**: 471, 1944.
- (3) WARBURG, O., F. WIND AND E. NEGELEIN. Klin. Wchnschr. **5**: 829, 1926.
- (4) EVANS, C. L., F. GRANDE AND F. Y. HSU. Quart. J. Exper. Physiol. **24**: 347, 1935.
- (5) HEYMANS, C. AND J. J., BOUCKAERT. Compt. rend. soc. biol. **119**: 324, 1935.
- (6) ENGEL, F. L., M. G. WINTON AND C. N. H. LONG. J. Exper. Med. **77**: 397, 1943.
- (7) RUSSELL, J. A., C. N. H. LONG AND F. L. ENGEL. J. Exper. Med. **79**: 1, 1944.

EFFECT OF BILATERAL DESTRUCTION OF THREE LATERAL CEREBRAL CORTICAL AREAS ON CORRECT CONDITIONED DIFFERENTIAL RESPONSES FROM GENERAL CUTANEOUS STIMULATION¹

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This fifth study on the function of the association cells of the cerebral cortex is concerned with the effects of destruction of one or more of three cortical areas previously investigated for sound effects (2, fig. 1, A, B, and C) on correct conditioned differential foreleg reflexes with 2 different sets of general cutaneous stimuli. In the first set of tests, the positive and negative conditioned stimuli consisted of stroking the left side of the back with a hand-brush once per second with the grain and once per second against the grain. In the second set of tests, based on timing, the positive and negative conditioned stimuli consisted of stroking the left side of the back with the grain once per second, and 3 times per second. Except for the use of general cutaneous stimuli, the general procedure and controls were the same as used earlier (2). A daily session consisted of 37 to 38 tests of which 25 were with the positive conditioned reflex and 12 or 13 were with the negative conditioned reflex, with an occasional reversal of this order. The first and usual daily session consisted of 5 positive to 2 negative conditioned tests up to and including the 20th positive test, then 5 negative tests, 4 positive, 1 or 2 negatives and one positive. To avoid differences of pressure of the brush as possibilities for the formation of positive and negative conditioned reflexes, the pressure of the brush was varied occasionally in some tests and sometimes in the same test, but ordinarily an attempt was made to keep the pressure of the brush uniform. Elimination of the cortex to the depth of the white matter was accomplished by extirpation and by thermo-coagulation with a high frequency current for 5 to 15 seconds depending on the depth of the sulci. Errors for the positive conditioned reflex were punished by shocking electrically the leg that was being tested, while errors for the negative conditioned reflex² were punished by a whip, a command *No!* or by both.

Bilateral elimination of cortical areas A and B (2, fig. 1). These areas (Woolsey and Walzl's first and second auditory afferent centers) were extirpated in dog 1 and coagulated in dogs 2 and 3. Both methods destroyed all of the cortex represented by the crosses and parallel lines in the above mentioned figure. A sufficient amount of the superficial optic radiations was injured to cause blindness, but in no instance did the damage reach the ventricles. The placing reflexes were present and the dogs could go up and down stairs rapidly by tapping

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² Dogs which understood the command *No!* acquired the negative conditioned reflex very quickly.

nose on steps. There were no motor symptoms and Marchi stained sections through the medulla disclosed no noteworthy degeneration in the pyramids.

TABLE 1
Conditioned stimuli: brush stroke, with and against grain

CORTICAL AREAS	BEFORE OPER. DIFF. FIRST APPEARED SESSION NO.	AFTER OPER. DIFF. FIRST APPEARED SESSION NO.	DAILY SESSIONS AFTER DIFF. WAS STANDARDIZED		TOTAL NUMBER OF TESTS		CHARACTER OF CORRECT CON. DIFF. RESPONSE
			Pos. C to I	Neg. I to C	Pos. C to I	Neg. I to C	
A and B Dog 1, E	Not tested	1st	25-0 12-0	0-12 1-24	112-2	1-100	Good
Dog 2, C	1st	1st	25-0 13-0	0-13 0-25	77-0	2-75	Good
Dog 3, C	1st	1st	25-0 13-0	0-13 0-25	88-0	2-62	Good
C Dog 4, E	Not tested	27th	25-0 13-0	0-12 0-25	760-4	412-51	Good
Dog 5, E	Not tested	25th	25-0 13-0	0-13 0-25	765-25	498-92	Good
Dog 6, E	1st	22nd	25-0 13-0	0-13 0-25	640-23	264-98	Good
B and C Dog 7, C	Not tested	None 30	23-2 11-1	11-1 24-1	652-57	339-30	No
Dog 8, E	1st	None 30	25-0 14-0	12-0 25-0	680-33	402-10	No
Dog 9, E	1st	None 30	25-0 13-1	13-0 25-0	631-73	336-30	No
Dog 10, C	1st	None 60	25-0 13-1	13-0 25-0	1417-20	782-19	No

Diff. = Correct conditioned differential response; Pos. = Positive conditioned reflex; Neg. = Negative conditioned reflex; Oper. = Operations; No. = Number; E. = Extirpated; C. = Coagulated; C to I = Ratio of correct to incorrect responses; I to C = Ratio of incorrect to correct responses.

Results from both sets of general cutaneous stimuli. Column 3 of tables 1 and 2 reveals that dogs 1, 2 and 3 produced correct conditioned differential responses for both the with and against stroke and the slow and fast stroke during the first session of tests which followed the operations, and only 2 to 4 additional sessions were required to make the perfect scores recorded in columns 4 and 5 of these

tables. The summary of all tests in columns 6 and 7 shows that these dogs had no difficulty after the lesions in making correct responses to either set of general

TABLE 2
Conditioned stimuli: brush strokes, slow and fast with grain

CORTICAL AREAS	BEFORE OPER. DIFF. FIRST APPEARED SESSION NO.	AFTER OPER. DIFF. FIRST APPEARED SESSION NO.	DAILY SESSIONS AFTER DIFF. WAS STANDARDIZED		TOTAL NUMBER OF TESTS		CHARACTER OF CORRECT CON. DIFF. RESPONSE
			Pos. C to I	Neg. I to C	Pos. C to I	Neg. I to C	
A and B Dog 1, E	Not tested	1st	25-0 13-1	0-12 1-24	113-0	14-63	Good
Dog 2, C	1st	1st	25-0 13-0	0-13 0-25	44-0	0-41	Good
Dog 3, C	1st	1st	25-0 13-0	0-13 0-25	63-0	1-50	Good
C Dog 4, E	Not tested	25th	25-0 13-0	0-12 0-25	688-0	317-79	Good
Dog 5, E	Not tested	2nd	25-0 13-0	0-13 0-25	88-6	9-56	Good
Dog 6, E	1st	3rd	25-0 13-0	0-13 0-25	162-1	22-78	Good
B and C Dog 7, C	Not tested	None 20	23-2 11-2	12-3 21-4	437-63	197-44	No
Dog 8, E	1st	None 30	25-0 13-0	13-0 25-0	700-2	416-2	No
Dog 9, E	1st	None 30	25-0 13-0	12-0 25-0	698-4	422-2	No
Dog 10, C	1st	None 50	25-0 13-0	13-0 25-0	1198-5	683-4	No

Abbreviations are listed and defined under table 1.

cutaneous analysers. In addition these dogs could respond correctly after the operations to alternate positive and negative conditioned tests for either set of stimuli when the interval between tests was of 3 seconds' duration.

Dogs 1, 2 and 3 were next tested for correct foreleg responses with 2 different sets of sound stimuli with entirely different results. They required 418 to 459 trials for the bell and cup analysers and a like number for the fast and slow bell

before any signs of correct responses appeared. All of these dogs had been perfectly trained for these sound tests before the operations and all were able to make perfect responses during the tests with either set of general cutaneous stimuli.

Bilateral elimination of cortical area C (2, fig. 1, C). This area of 10 mm. or less in diameter is situated at the base of the ectosylvian and coronal gyri, being separated from area B above by a conspicuous blood vessel. It represents Tunturi's third auditory projection center and includes all or a large part of Woolsey's second tactile afferent center.

Area C was extirpated in dogs 4, 5 and 6 with no damage to area B and very little to the white matter below. The sulci in this region are superficial and too deep destruction can result in motor symptoms. The dogs were not blinded by the lesions. The placing reflexes were present and all parts of the body were sensitive to general cutaneous stimuli. Motor symptoms were absent and no noteworthy degeneration was observed in the pyramids of Marchi stained sections through the medulla.

Results from both sets of general cutaneous stimuli. Before operation, column 2 of tables 1 and 2 shows that dog 6 acquired correct conditioned differential responses for each set of general cutaneous stimuli during the first session of tests. It can also be stated that this dog made only 2 errors in each of the sessions and they were for the first 2 negative conditioned tests. Dogs 4 and 5 were not previously trained with general cutaneous stimuli, but had acquired correct responses for 2 sets of sound analysers.

The unilateral lesion permitted immediate appearance of correct responses with both sets of general cutaneous stimuli.

After the bilateral lesions the positive conditioned reflex returned immediately and unaltered. On the other hand, as shown by column 3 of tables 1 and 2 correct conditioned differential responses were not established for the with and against stroke with dogs 4, 5 and 6 until the 27th, 25th and 22nd sessions (987, 922 and 836 trials) or for the slow and fast stroke until the 25th, 2nd and 3rd sessions of 37 or 38 tests. The better showing of dogs 5 and 6, but not 4, with the slow and fast stroke might be attributed to the fact that these tests were not started until perfect scores had been obtained for the with and against stroke. It should also be mentioned that after correct responses had appeared with these general cutaneous analysers for dogs 4 to 6, it required 4 to 8 more sessions of tests than would be necessary for normal dogs to produce the perfect scores recorded in columns 4 and 5 of tables 1 and 2. The great difficulty that these dogs had in acquiring association circuits that were capable of evoking correct conditioned differential responses with these general cutaneous stimuli is well illustrated in the summary of all tests given in columns 6 and 7.

The following additional results can be recorded for dogs 4, 5 and 6 after area C had been destroyed on both sides: All were ultimately able to make perfect responses to a series of alternate positive and negative conditioned tests for either set of general cutaneous stimuli when the interval between tests was of 3 seconds' duration. During the time in which the lesions prevented correct responses for

the general cutaneous stimuli, correct responses were always obtainable with 2 different sets of sound stimuli. It became obvious as a daily session of differential tests progressed that the situational tension which resulted from inability to withhold foreleg response during the negative conditioned tests was gradually weakening and slowing up all conditioned foreleg responses during the session. The sensory side of the positive reflex also became more generalized. Instead of the reflex being elicited only from brushing the left side of the back or from both sides of the back in about 50 per cent of the dogs, it could be evoked equally well from the left shoulder, left hip, belly, tail, or from tapping the left side of the back with the hand.

Elimination of cortical areas B and C (2, fig. 1). These areas were extirpated with dogs 8 and 9 and coagulated for dogs 7 and 10 with no damage to area A and very little to the white matter below. All of the dogs were blinded by the operations, but their placing reflexes were good as was their general sensory response from all parts of the body. No motor symptoms were noted and Marchi stained sections through the medulla demonstrated no noteworthy degeneration in the pyramids. All could go up and down stairs rapidly by tapping nose on the steps.

Results from both sets of general cutaneous stimuli. It is apparent from column 2 of tables 1 and 2 that dogs 8, 9 and 10 required but one session of tests before the operations to acquire correct conditioned differential responses for either set of general cutaneous stimuli. The only errors for these sessions were positive responses for 2 or 3 negative conditioned tests. Dog 7 was trained for the sound analysers before the operations, but not for the general cutaneous analysers.

None of the dogs had any difficulty after the unilateral lesion in making correct responses with either set of general cutaneous stimuli.

After the bilateral lesions the positive conditioned reflex appeared unaltered with the first tests. On the other hand, column 3 of tables 1 and 2 discloses that correct conditioned differential responses were not acquired with dog 7³ or re-established with dogs 8, 9 and 10 during 30 daily sessions (1120 to 1140 trials) with either set of general cutaneous stimuli. Dog 10 was given 30 additional sessions or a total of 2238 trials for the with and against stroke and 20 additional sessions or a total of 1890 trials for slow and fast stroke without any sign of correct responses. It can also be stated that a number of the later tests were made during the 5th month following the last operation. The poor scores for daily sessions shown in columns 4 and 5 of tables 1 and 2 are from the last 2 daily sessions, but are representative after the lesions. The summary records of columns 6 and 7 clearly demonstrate that failure to make correct responses after the lesions is due to absence of correct inhibition during the negative conditioned tests. The 58th session with dog 10 for the with and against stroke was unusual in that instead of resulting in 38 positive foreleg responses, there were 8 failures

³ On account of the appearance of convulsions dog 7 was only given 20 sessions of tests with the slow and fast brush stroke.

to respond, 5 being for the positive conditioned tests and 3 for the negative. However, all tests for the 59th and 60th sessions resulted in positive responses.

Throughout the entire interval when correct responses were not obtainable from general cutaneous stimuli—correct conditioned differential responses could always be evoked from 2 different sets of sound stimuli. As noted for the effects of bilateral destruction of area C, the tension resulting from inability to make correct responses for the tests, slowed up and weakened all of the foreleg responses, together with increasing the area greatly from which stimulation was effective.

In addition the writer had 3 dogs in which bilateral destruction of cortical areas A, B and C abolished each dog's ability to make correct responses with either set of general cutaneous analysers for over 1100 tests.

DISCUSSION. Particular attention is called to the fact that correct conditioned differential responses with 2 different sets of auditory stimuli were always obtainable during the time that correct responses could not be evoked from either set of general cutaneous stimuli as a result of bilateral destruction of cortical areas C, or B and C.

With these general cutaneous tests, bilateral deletion of area C was equivalent to bilateral destruction of areas A and B for the auditory tests, in that association circuits could ultimately be established for making correct conditioned differential responses. Also bilateral elimination of cortical areas B and C with the general cutaneous tests was equivalent to bilateral destruction of areas A, B and C with the auditory tests (2) in that new association circuits were not established during a long series of trials.

It is not known where or how elimination of cortical areas C or B and C disrupted the usual association circuits for making correct responses with these general cutaneous stimuli. Area C, situated at the base of the anterior ectosylvian gyrus, is a region from which Marshall, Woolsey and Bard, and Adrian have obtained voltage changes from tactile stimulation of the forepaw or the feet. It also includes Woolsey's second afferent somatic center, which is said to receive tactile impulses from all parts of the body. Tunturi has recorded voltage changes from this area from tonal stimulation and from localized stimulation of the organ of Corti. Garol's area 3s for suppression of electrical activity of the cat's motor cortex is also in this locality. Tower has obtained extrapyramidal inhibition of motor activity from stimulation of large portions of the sylvian and ectosylvian gyri. It should also be mentioned that area B included a narrow strip of the coronal gyrus, a terminal for general cutaneous sense.

Since the process of training for these correct conditioned differential responses involved shocking the foreleg for failures to flex the foreleg for the back-foreleg conditioned reflex, it is possible that the association circuits for eliciting these general cutaneous responses may include projections to the foreleg sensory cortex as well as to the back sensory cortex. Stroking the back with the grain and shocking the foreleg apparently means one and the same thing to these dogs.

Pavlov quotes an experiment by Krasnogorsky in which the left coronal and anterior ectosylvian gyri were removed after establishment of a very generalized

conditioned salivary reflex which could be evoked by acid from any part of the body. On the 10th day following the operation the reflex was obtained, but only from the trunk region of the right side. After the 90th day following the operation the reflex returned to the right side of the body in the following order: shoulder, pelvis and paws. During the interval in which this reflex could not be elicited, the dog was said to be in a state of sleepiness due to internal inhibition.

Although bilateral destruction of cortical areas B and C permitted immediate and unaltered return of the positive conditioned reflex, the situational tension which developed from inability to make correct conditioned differential responses caused the foreleg movement to become slower and weaker as a session of tests progressed. Also the place of effective stimulation was no longer confined to the left side of the back or in some cases to both sides of the back, but could be evoked equally well from the left sides of the shoulder, belly, hip, tail, or from tapping the left side of the back with the hand.

SUMMARY AND CONCLUSIONS

Bilateral elimination of cortical areas A and B reported previously to be very effective in disrupting the usual circuits for eliciting correct conditioned differential responses with sound, permitted the circuits for evoking correct responses with general cutaneous sense to function perfectly.

Bilateral deletion of cortical area C disrupted completely the association mechanism for making correct conditioned differential responses with 2 sets of general cutaneous stimuli. Re-establishment or establishment of correct responses required 836 to 987 trials for the with and against stroke and a lesser number, later, for the slow and fast stroke.

Bilateral destruction of cortical areas B and C prevented formation of new association circuits capable of producing correct responses with both sets of general cutaneous analysers during a very long and intensive period of testing.

Correct conditioned differential responses from 2 different sets of sound stimuli could always be obtained during the time in which the lesions abolished correct responses from the general cutaneous stimuli.

Unilateral destruction of areas B and C produced no effect on correct responses from general cutaneous stimuli. Likewise, none of the bilateral lesions delayed or altered the positive conditioned reflex. Some situational tension effects, however, were noted for the reflex in the discussion.

Inability to withhold foreleg flexion during the negative conditioned tests was the chief defect noted from destruction of cortical areas C, or B and C. This involves *correct* inhibition.

REFERENCES

- (1) ADRIAN, E. D. *J. Physiol.* **100**: 159, 1941.
- (2) ALLEN, W. F. *This Journal* **144**: 415, 1945.
- (3) GAROL, H. W. *J. Neuropath. and Exper. Neurol.* **1**: 139, 320, 1942.
- (4) MARSHALL, WOOLSEY AND BARD. *J. Neurophysiol.* **4**: 1, 1941.
- (5) MUNK, H. *Über die Functionen der Grosshirnrinde.* Berlin, 1890.
- (6) PAVLOV, I. P. *Conditioned reflexes.* Transl. by C. V. Anrep, 1927.

- (7) ROTHMANN, M. Monatsch. f. Psychol. u. Neurol. **36**: 319, 1914.
- (8) TOWER, S. S. Brain **59**: 408, 1936.
- (9) TUNTURI, A. R. This Journal **141**: 397, 1944; Ibid. **144**: 389, 1945.
- (10) WOOLSEY, C. N. Fed. Proc. **5**: 116, 1946.
- (11) WOOLSEY AND WALZL. Bull. Johns Hopkins Hosp. **71**: 315, 1942.

THE METABOLISM OF HISTAMINE AND ADENYLIC COMPOUNDS IN THE EMBRYO

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Histamine and adenosine have come to be recognised as the two most powerful vasodilator substances among the "metabolites" of the body. Blood and tissues of all the animals so far investigated contain measurable amounts of these substances although in greatly varying concentrations. While, for example, the kidneys of most animals contain only traces of histamine, the lungs and the intestine may have as much as 15-30 μg per gram. On the other hand, the intestine contains about 8 times less biologically active adenylic compounds than the muscle. It was shown recently (Anrep, Ayadi, Barsoum, Smith and Talaat, 1944) that considerable amounts of histamine in a conjugated and inactive form can be excreted in the urine. This excretion greatly depends on the nature of the food and in carnivora it is especially high. In the dog after administration of meat it may reach 6 mgm. of histamine base per day. The source of this histamine is mainly exogenous since its excretion runs roughly parallel with the amount of histamine present in the food. However, on a histamine free diet and in prolonged starvation the excretion of histamine, although greatly reduced, is not completely abolished; the tissue histamine does not disappear and the concentration of histamine in the blood remains almost constant. These observations suggest that histamine as a normal constituent of tissues may be in part of an endogenous origin. Similar considerations apply even to a greater extent to adenylic compounds, which form an integral part of the general nucleoprotein metabolism. With the view of testing this possibility I undertook, at the suggestion of Prof. G. V. Anrep, to investigate the metabolism of histamine and of adenylic compounds in the developing egg and to compare it with that of the mammalian foetus. For this purpose the hen and the rat were selected as convenient animals because the duration of their embryonic development is approximately the same, the incubation period of the chick being on the average 21 days and the gestation time of the local rat 21-22 days. No other points of similarity should be, of course, looked for since the nutrition of the chick is, with the exception of oxygen, entirely endogenous, while that of the rat's foetus depends on the placenta. The rate of growth of the chick's embryo is much more rapid than that of the rat, the weight of the freshly hatched chick of the local breed averaging about 29 grams, that of the newly born rat about 5 grams.

METHODS. The observations on the eggs were made on two separate batches of about 200 eggs each. Since the hatching period of similarly incubated eggs may differ by as much as 24 hours, all the results are presented as averages of

estimations made on several eggs of the same length of incubation. Up to the 9th day of development the complete separation of the embryo from the remainder is not reliable. It was, therefore, preferred to take each embryo during these early stages together with 2 cc. of the fluid remainder. This introduced no error in the determination of the total amount of the two active principles present in the embryo, since at this stage the remainder contains none, but it made it impossible to calculate their concentration per gram of embryo. Beginning from the 9th day of incubation the separation of the embryo from the rest presented no difficulty and the analyses could be carried out separately in the embryo and the total remainder comprising the yolk, egg white, amnion and the embryonic membranes. The shell and the shell membrane were always discarded.

In the early stages of incubation the embryos were analysed together in batches of 10 to 20; beginning from the 13th day, in batches of 3 to 5 and later, singly, not less than 10 embryos being used for each point.

For the rats, two separate batches of females, 220 to 250 grams each, were kept for 24 hours together with males and then separated. At different days of gestation the embryos of several rats were freed from all their membranes and weighed together. Their total histamine and adenosine content was then determined and calculated per embryo and per gram of fresh tissue. In view of possible autolytic destruction of adenylic compounds (Buell, Strauss and Andrus, 1932) the chick's and rat's embryos were immediately placed in a weighed container with a 10 per cent solution of trichloroacetic acid and rapidly minced to a fine pulp, the whole procedure taking less than two minutes. The weight of the embryo was then determined by a second weighing of the container.

The histamine and the adenylic compounds were determined by the method of Barsoum and Gaddum (1935), histamine being assayed on the atropinised guinea-pig ileum and the adenylic compounds on the rectal coecum of the fowl. In the beginning of the investigation the extracts were divided into two parts for the determination of the free and total histamine. For the total histamine, the extracts were, as recommended by Barsoum and Gaddum, hydrolyzed in acid while for the free histamine they were assayed on the ileum without preliminary hydrolysis. Since, however, no conjugated histamine could be detected in any of the samples, separate estimations of the free and total histamine were abandoned and all the assays were made after hydrolysis of the extracts in acid.

All the results given in this communication represent arithmetical averages; a more detailed statistical analysis is omitted since the standard deviations from the respective averages were in every case insignificant.

The developing egg. Histamine metabolism. It was ascertained as a preliminary to this investigation that non-fertilised eggs are completely free from histamine and similarly active substances. In fact, extracts of the yolk evoke a negligible relaxation of the ileum which is, however, not strong enough to mask even so small an amount of histamine as 0.02 $\mu\text{g/cc}$. The results of histamine estimation at different stages of the incubation are given in table 1.

In this series of observations, the chicks, after 21 days of incubation, were taken immediately after hatching. On the 22nd and 23rd days they were killed 24 and 48 hours respectively after hatching. The progressive increase of the histamine content of the embryo is rendered more obvious in figure 1a. The increase is at first very slow. It becomes faster after the 12th day of incubation and reaches its maximum during the period of the greatest growth of the embryo. After hatching and in absence of food the increase of the histamine content continues although the weight of the chick somewhat declines. The increase of histamine is not entirely accounted for by the growth of the embryo since its concentration per gram of tissue also progressively rises during the incubation, although in a less precipitate manner than the total histamine. Nevertheless, between the 13th and the 19th day the histamine concentration of the embryo increased tenfold and continued to rise after hatching (fig. 1b).

In the remainder of the egg, the histamine can be detected in negligible concentrations beginning from the 9th day. Its distribution between the different parts of the remainder, the membranes, amnion, clear fluid and the yolk, is approximately uniform. It is likely that the histamine of the remainder is due to diffusion from the embryo. The entire histamine of the developing egg is present in a free form, at no stage of the development was any conjugated histamine detected either in the embryo or in the remainder. Additional evidence in support of the active principle being histamine was provided by the use of histaminase and by the method of Barsoum and Gaddum (1935) and of Barsoum and Smirk (1938) of selective paralysis of the guinea-pig ileum with large doses of histamine. All the extracts were completely inactivated by incubation with a strong preparation of histaminase. After treatment of the ileum with a strong solution of histamine diphosphate (0.5 $\mu\text{g}/\text{cc.}$) it ceased to contract in response to the administration of the extracts although its reaction to other contractile agents remained unimpaired.

Adenylic compounds. Biologically active adenylic compounds include adenosine, adenylic acid and possibly other related substances. The activity of adenylic acid is about $\frac{2}{3}$ that of adenosine. Since, however, there is no easy way of separating these compounds from each other, all the results of our assays are expressed in terms of adenosine. The details of the method are described by Barsoum and Gaddum (1935) who made use for the quantitative assay of adenylic compound of their strong resistance to alkali hydrolysis. Additional support in favour of the active substances belonging to the group of adenylic compounds was provided by the fact that the extracts were completely inactivated by hydrolysis with acid.

Non-fertilised eggs contain no adenosine or similarly acting substances. Their gradual increase during the growth of the embryo is shown in table 2 and figure 1a. It can be seen that the production of adenylic compounds is as conspicuous as that of histamine. The two substances run approximately parallel to each other except that the rapid increase in histamine is delayed by 2-3 days as compared with that of adenosine. The concentration of histamine per gram of tissue gradually increases during the whole period of the embryonic

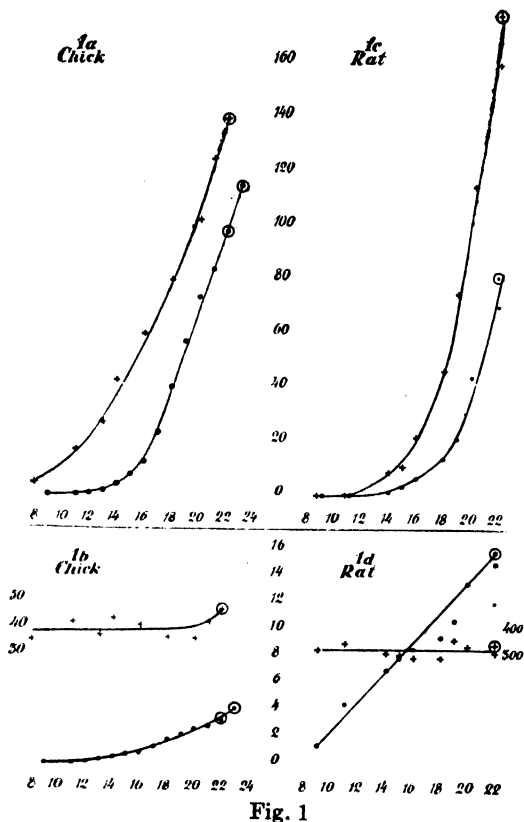


Fig. 1

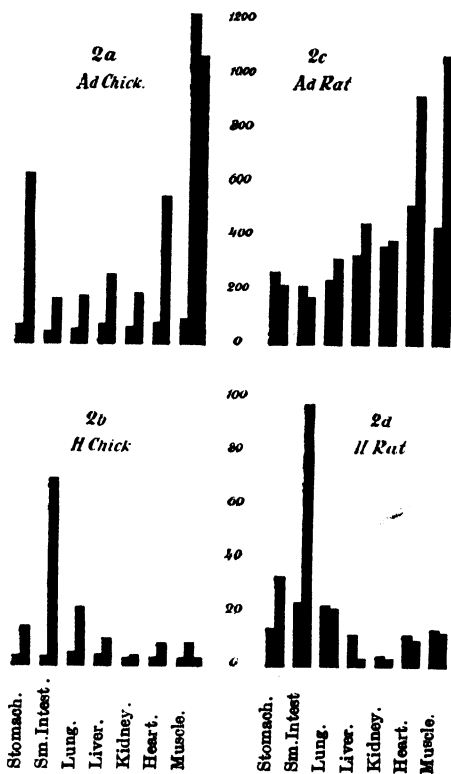


Fig. 2

Fig. 1. Increase of histamine and of adenosine in the developing embryo of the chick (fig. 1a) and of the rat (fig. 1c).

Concentration of histamine and of adenosine per gram of fresh tissue in the developing embryo of the chick (fig. 1b) and of the rat (fig. 1d).

Histamine before birth is shown by points, after birth by encircled points: adenosine, before birth, by crosses and after birth by encircled crosses.

The abscissae of the four figures show days of development. The ordinate between 1a and 1c shows histamine in micrograms; the same ordinate multiplied by 10 shows adenosine in micrograms.

The ordinate between 1b and 1d shows histamine in micrograms; the ordinate for the adenosine of the chick is given on the left hand side of figure 1b and that of the rat on the right hand side of figure 1d.

Fig. 2. Comparison of histamine and of adenosine concentration in the organs of the newly born chick and rat with that of the respective adults. In each pair of rectangles the one on the left is for the newly born animal and on the right for the adult. There are three rectangles for the skeletal muscle of the hen, the first is for the newly hatched chick, the second and the third for the red and white muscle of the adult hen respectively.

The ordinate for adenosine and for histamine are in micrograms per gram of fresh tissue.

Fig. 2a. Adenosine in the organs of the chick and hen.

Fig. 2b. Histamine in the organs of the chick and hen.

Fig. 2c. Adenosine in the newly born and in the adult rat.

Fig. 2d. Histamine in the newly born and in the adult rat.

TABLE 1

Increase in the histamine content of the developing chick's embryo and of the remainder of the egg

Histamine is given in equivalents of histamine diphosphate in micrograms.

DAYS OF INCUBATION	NUMBER OF EMBRYOS ANALYZED	EMBRYO			REMAINDER		
		Average weight of single embryo	Histamine per embryo	Histamine per gram of embryo	Average volume of remainder	Histamine in remainder	Histamine per cc. of remainder in
		grams	µg.	µg.	cc.	µg.	µg.
4	36		traces				
9	20	2.0	0.1	0.05	30.8	0.9	0.03
11	18	3.1	0.4	0.13	30.0	0.9	0.03
12	16	4.2	0.9	0.21			
13	20	6.2	1.6	0.26	22.0	1.1	0.05
14	18	8.2	4.2	0.51			
15	16	12.5	7.8	0.62			
16	17	16.0	12.0	0.75	14.0	1.3	0.09
17	11	18.0	23.4	1.30			
18	10	22.7	40.5	1.78	6.7	1.0	0.15
19	15	25.1	57.4	2.28			
20	19	28.4	73.8	2.60			
21 [†] (just hatched)	15	29.5	84.5	2.86			
22 [‡] (24 hours after hatching)	11	28.9	98.2	3.40			
23 (48 hours after hatching)	15	28.2	115.0	4.10			

TABLE 2

Increase in the biologically active adenylic compounds of the developing chick's embryo and of the remainder of the egg

The adenylic compounds are given in equivalent of adenosine in micrograms.

DAYS OF INCUBATION	NUMBER OF EMBRYOS ANALYZED	EMBRYO			REMAINDER		
		Average weight of single embryo	Adenosine per embryo	Adenosine per gram of embryo	Volume of remainder	Adenosine in remainder	Adenosine per cc. of remainder
		grams	µg.	µg.	cc.	µg.	µg.
8	16	1.4	52	37.0	29.5	40	1.4
11	18	4.1	173	42.2	28.0	60	2.1
13	9	6.9	270	39.2	22.7	79	3.5
14	14	9.2	420	45.6	20.0	100	5.0
16	16	14.5	608	42.0	14.8	116	7.8
18	11	20.8	810	39.0	7.1	127	17.9
20	9	26.6	1,040	39.2			
21	12	28.9	1,260	43.6			
22 (24 hours after hatching)	8	28.2	1,400	49.6			

development, while that of adenylic compounds remains approximately constant (fig. 1b). The increase of the adenylic compounds is, therefore, entirely accounted for by the growth of the embryo.

In the remainder of the egg the adenylic compounds gradually increase in total amount and in concentration. The increase is more conspicuous than that of histamine and it is unlikely that it can be entirely accounted for by a simple diffusion from the embryo.

The mammalian foetus. The rats were killed by chloroform in batches of 5 to 12 beginning from the 9th day of gestation. The embryos were freed from their membranes, weighed together and analysed for histamine and adenylic compounds. The results obtained are given in table 3 and figure 1c.

It can be seen that the rate of histamine production by the rat's embryo is approximately the same as in the chick. Considering, however, that the average

TABLE 3

Increase of histamine and of biologically active adenylic compounds in the developing rat's embryo

The histamine is given in equivalents of histamine diphosphate and the adenylic compounds in equivalents of adenosine in micrograms.

DAYS OF GESTATION	NUMBER OF EMBRYOS ANALYZED	AVERAGE WEIGHT OF SINGLE EMBRYO	HISTAMINE		ADENOSINE	
			Per embryo	Per gram of embryo	Per embryo	Per gram of embryo
		grams	μg.	μg.	μg.	μg.
9	26	0.006	0.01	1.7	2	334
11	18	0.02	0.08	4.0	7	350
14	26	0.27	1.90	7.0	86	318
15	27	0.35	2.8	8.0	109	314
16	24	0.71	6.0	8.5	220	310
18	22	1.49	14.0	9.4	454	305
19	8	2.05	21.7	10.6	754	368
20	23	3.30	45.3	13.7	1,150	348
22	12	4.65	70.2	15.1	1,600	344
22 (born)	13	5.10	80.6	15.8	1,778	349

weight of the rat at birth is about one-sixth that of the chick, the increase in the histamine concentration per gram of tissue is in the rat's embryo much more rapid (fig. 1d). As regards adenylic compounds their gradual increase runs, as in the chick, roughly parallel to that of histamine but in advance of it by about two days. Their concentration per gram of tissue, again as in the chick's embryo, remains approximately constant throughout the period of gestation (fig. 1d) except that in the mammalian foetus it is about 8 times greater.

Distribution of histamine and of adenylic compounds in the embryo. The considerable increase of histamine and of biologically active adenylic compounds in the embryo raises the question as to whether these substances are uniformly distributed between the different tissues of the embryo or selectively increased in some particular organs.

Twenty newly hatched chicks and twenty newly born rats were killed in batches of 5. The identical organs in each batch were dissected, weighed together and jointly extracted with trichloracetic acid. One half of each extract was used for the determination of histamine and the other half for that of adeno-

sine. For comparison, the concentration of these substances was also determined in the organs of 7 adult hens and 15 adult rats of the same respective breeds. The organs of each adult animal were analysed separately and not in batches. The averages for each organ are given in table 4 and are made more evident in figure 2.

It must be stated in this respect that the differences between the determinations of histamine and adenosine in the individual batches of 5 embryos each and in the single animals were rather considerable. The maximal standard

TABLE 4

Distribution of histamine and of biologically active adenylic compounds in the organs of the newly born chick and rat and the respective adults

The average weight of the chicks (20) was 29.5 grams, of the adult hens (7) 1250 grams, of the newly born rats (20), 5.1 grams of the adult rats (15) 232 grams. The maximal standard deviations for the histamine and adenosine determinations in the newly born animals did not exceed ± 20 per cent and in the adult animals ± 25 per cent. Histamine (as diphosphate) and adenylic compounds (as adenosine) are given in micrograms per gram of fresh tissue.

	HEN				RAT			
	Histamine		Adenosine		Histamine		Adenosine	
	Chick	Hen	Chick	Hen	Ratlet	Rat	Ratlet	Rat
Stomach.....	2.6	14.7	66	630	15.4	34.0	270	225
Small intestine.....	2.3	76.3	46	165	24.5	98.0	210	180
Lung.....	5.1	22.3	50	172	24.3	23.0	245	325
Liver.....	3.9	9.7	75	268	11.8	3.3	342	450
Kidney.....	2.5	3.9	63	188	3.8	3.2	375	385
Heart.....	2.4	9.2	84	550	12.2	10.2	250	935
red }		9.1	92	1,220	14.5	14.0	440	1,065
Muscle white }	2.1	1.9		1,070				

deviations from the averages given in table 4 were as great as ± 20 per cent for the embryos and ± 25 per cent for the adults. These differences are only in part due to the inherent error of biological assays in general; they are mainly due to the individual variations between different animals. On account of this, when comparing the estimations of histamine and adenylic compounds in the embryo and in the adult only those differences which exceed 100 per cent can be considered as significant.

In the newly born chick the histamine and the adenylic compounds are much more uniformly distributed than in the hen. In the adult bird the concentration of both substances is increased in almost all the organs but to a very different extent. As regards histamine, the increase is most striking in the lung and in the digestive tract, especially in the small intestine. There is no increase in the kidney and in the white muscle. An interesting difference is presented by the red and white muscle. In the newly hatched chick the morphological

distinction between the two kinds of muscle is not yet apparent; all the chick's muscles were, therefore, analysed together giving an average histamine concentration of $2.1 \mu\text{g}$ per gram. In the adult, the histamine concentration in the white muscle remained approximately the same ($1.9 \mu\text{g}/\text{gram}$), while that in the red muscle increased to an average of $9.1 \mu\text{g}$. The heart gave in this respect the same results as the red muscle.

The distribution of adenylic compounds amongst the different organs of the chick is also more uniform than in the adult hen. In the latter the adenosine concentration is increased in all organs and, as would be expected, the increase is more marked in organs containing a large amount of muscle tissue, the ventriculum, the heart and the skeletal muscle. No significant difference in the adenosine content is found in red and white muscle.

In the young rat the concentration of the two substances in the different organs is considerably greater than in the chick and their distribution is not as uniform. In the adult rat the histamine increases only in the digestive tract. In the kidneys it remains very low, while in the liver there is a significant diminution. It should be mentioned in this respect that the liver of the rat, unlike that of many other animals, is known to be poor in histamine; its concentration is always less than in the muscle.

As regards adenylic compounds there is no increase in their concentration in the organs of the adult rat except in the skeletal muscle and in the heart. The adenosine of the rat's stomach unlike that of the hen's ventriculum remains unchanged, probably because it contains much less muscle tissue.

SUMMARY

The observations described in this communication prove that histamine and biologically active adenylic compounds (adenosine) are endogenously produced by the chick's embryo during its development. The production of both substances runs approximately parallel to the growth of the embryo, except that the maximal increase of the histamine is delayed by 2-3 days as compared with that of adenosine.

The concentration of histamine per gram of embryo increases during the whole course of the embryonic development. This shows that some qualitative change is taking place in the composition of the tissues as regards histamine. On the other hand, the concentration of adenosine remains approximately constant throughout the period of incubation so that the total increase of adenosine is entirely accounted for by the growth of the embryo.

The distribution of histamine and adenosine amongst the different organs of the chick's embryo is approximately uniform. The selective distribution of these substances in the adult hen takes place almost entirely during the post-natal life. Histamine and adenosine increase in all the organs of the adult but to a different extent. The maximal increase of histamine is observed in the digestive tract and in the lung; there is no increase in the kidney and in the white muscle. The maximal increase of adenosine occurs in the ventriculum, heart and in the skeletal muscle, both white and red, i.e., in all those organs which are rich in muscle tissue.

The metabolism of histamine and of adenosine in the rat's foetus, although its nutrition is exogenous through the placenta, is surprisingly similar to that of the chick. The duration of the embryonic development of the two animals is approximately the same. The total histamine content of the newly hatched chick is on the average 85 μ g and that of the newly born rat 80 μ g. The respective amounts of adenosine are 1.3 mgm. and 1.7 mgm. In the rat, as in the chick, the maximal increase of histamine is delayed by about two days as compared with that of adenosine. The concentration of histamine gradually increases in the chick and in the rat's foetus during their development. On the other hand the concentration of adenosine remains in both approximately constant.

All these points of similarity suggest that in the rat as in the chick, the two substances are produced as a result of the metabolic processes taking place in the embryo itself and are not supplied through the placenta in a ready made form.

The distribution of histamine and adenosine amongst the different organs of the rat's embryo is much more uniform than in the respective adult animals. In the post-natal life the histamine concentration increases in the rat as in the hen mainly in the digestive tract, while that of adenosine mainly in the organs rich in muscle tissue.

I should like to express my thanks to Prof. G. V. Anrep and to Dr. G. S Barsoum for the constant help and advice which they gave me during the course of my work.

REFERENCES

- ANREP, G. V., M. S. AYADI, G. S. BARSOUM, J. R. SMITH AND M. TALAAT. *J. Physiol.* **103**: 155, 1944.
BARSOUM, G. S. AND J. H. GADDUM. *J. Physiol.* **85**: 1, 1935.
BARSOUM, G. S. AND F. H. SMIRK. *Clin. Science* **2**: 337, 1936.
BUELL, M. N., M. B. STRAUSS AND E. C. ANDRUS. *J. Biol. Chem.* **98**: 645, 1932.

THE INFLUENCE OF STEROIDS ON THE RESTORATION OF HYPERTENSION IN HYPOPHYSECTOMIZED RATS¹

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In previous reports, we have demonstrated that hypophysectomy causes a partial or total abolition of experimental renal hypertension in rats (1) and that the administration of purified adrenocorticotrophic hormone will restore the hypertension to the pre-hypophysectomy level (2). This would suggest that adrenal cortex insufficiency is the principal cause for the fall of blood pressure following removal of the pituitary gland. Goldblatt (3), I. H. Page (4), Collins and Wood (5) and several others have shown that experimental renal hypertension in the dog is abolished by bilateral adrenalectomy, and that partial restoration of the hypertension follows treatment with salt and adrenal cortical extracts.

That the absence of the adrenal cortex may interfere with the humoral mechanism of renal hypertension is suggested by the observations of Goldblatt (3) and of Houssay and Dexter (6) that the concentration of renin substrate in the plasma is markedly reduced in the majority of dogs after adrenalectomy.

Gaudino (7) found that renin substrate almost disappears from rats' blood about 11 days after bilateral adrenalectomy, and is restored by subsequent treatment of the animals with desoxycorticosterone. This sequence of events was confirmed on dogs by Collings, Ogden and Taylor (8). In humans with Addison's disease the blood pressure may be restored to normal with adequate replacement therapy, and overtreatment with desoxycorticosterone and salt may even result in hypertension (9). Gaudino concludes that the adrenal cortex mediates in the production of renin substrate, possibly by stimulating its synthesis in the liver.

When renal hypertension of short duration is established in rats and then abolished by removal of the pituitary gland, the animal becomes a sensitive test object for the blood pressure raising properties of adrenal cortical hormones. Leatham and Drill (10) were unable to prevent completely the drop of systolic blood pressure following hypophysectomy in normal rats with the use of adrenal cortical extract or desoxycorticosterone, and they suggested that some factor other than adrenal insufficiency accounted for this fall. This is analogous to our experience presented below, in which similar replacement therapy failed to restore completely the original level of hypertension. A like observation was made by Dell'Oro (11), who removed both adrenal glands from hypertensive rats and then attempted to restore the hypertension by replacement therapy.

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On the other hand, when purified adrenocorticotrophic hormone is given after hypophysectomy (2), or when accessory adrenal cortical tissue hypertrophies after bilateral adrenalectomy (11), then the hypertension is completely restored and in some cases even exceeds the original level. This may be interpreted in one of two ways: Either the replacement therapy by adrenal cortical extract is inadequate as to dosage or method of administration, or there is some factor other than desoxycorticosterone elaborated by the adrenal cortex that exerts an influence upon the blood pressure.

Inasmuch as some steroids related in structure to those of the adrenal cortex are known to have some effects upon life maintenance or electrolyte metabolism following adrenalectomy (12), we have endeavored to test both types of hormones for their blood pressure restoring properties.

METHODS. Renal hypertension was induced by a partial ligation of the left renal artery, and the systolic blood pressure was measured three or four times a week on the warmed, unanesthetized rats by the tail plethysmograph method (13).³ From 3 to 16 weeks later, when the blood pressure had stabilized at a hypertensive level, total hypophysectomy or bilateral adrenalectomy was done. There was always a pronounced fall in blood pressure, usually to normal levels. The adrenalectomized animals were maintained on one percent salt in the drinking water, and all animals were kept in a temperature controlled room. After the systolic pressure had attained a plateau, commonly from two to four weeks after surgery, daily injections of desoxycorticosterone, adrenal cortical extract, progesterone, testosterone or estradiol were given as indicated in the tables. After cessation of treatment, the blood pressure was followed daily until it had resumed its pre-injection level.

RESULTS. The essential data for each animal are presented in tabular form, together with the mean values for each group. From table 1 it may be seen that 1 mgm. of desoxycorticosterone in oil given daily for 6 days produced an average rise of 18 mm. which persisted for about 4 days. This is a small rise when compared to the average fall of 46 mm. resulting from the hypophysectomy. When the dosage was doubled and was administered for 10 days, the average rise was 25 mm., and the increased blood pressure persisted twice as long after completing the injections. In only three instances (all in the first group) was the original level of hypertension restored, and these were all cases in which hypophysectomy had resulted in the smallest decline of blood pressure. In

³ The accuracy of the measurement of blood pressure in the rat by a tail plethysmograph has recently been questioned (10). We have checked the pressor responses resulting from the injection of drugs by both the indirect tail plethysmograph and by direct cannulation of the carotid artery, using a small Herthle manometer. The tail pressures are lower than the carotid pressures, but the rises produced by a given dosage of a pressor substance appear to be the same when recorded by either method. The fact that the tail pressure is lower than the absolute pressure should not affect the validity of the results. It has been claimed (11) that in some circumstances the warming of rats results in a rise of blood pressure. Our animals were exposed for 10 minutes to a temperature of 40°C. in a thermostatically controlled ventilated box and then placed in open screen holders in an environment of 37°C. during the measurement of blood pressure.

three animals, the administration of 1 ml. of adrenal cortical extract (Upjohn) in daily divided doses produced an effect equivalent to that of 1 mgm. of desoxycorticosterone.

TABLE 1

The effect of hypophysectomy and subsequent treatment with desoxycorticosterone or adrenal cortical extract upon the blood pressure of rats with renal hypertension

BEFORE HYPO- PHYSECTOMY	AFTER HYPO- PHYSECTOMY	DESOXYCORTICOSTERONE IN OIL (i.m.)			
B.P. (1)	B.P. (2)	Dose (3)	B.P. (4)	Change (5)	Period of decline (6)
					<i>Days</i>
181	120	1 mgm./day for 6 days	138	+18	6
180	163	1 mgm./day for 6 days	175	+12	4
170	129	1 mgm./day for 6 days	142	+13	3
240	160	1 mgm./day for 6 days	178	+18	6
207	150	1 mgm./day for 6 days	177	+27	4
163	141	1 mgm./day for 6 days	165	+24	2
203	164	1 mgm./day for 6 days	181	+17	6
151	108	1 mgm./day for 6 days	127	+19	6
152	100	1 mgm./day for 6 days	112	+12	
150	135	1 mgm./day for 6 days	151	+16	
<i>183</i>	<i>137</i>		<i>155</i>	<i>+18</i>	<i>4</i>
175	141	2 mgm./day for 10 days	168	+27	6
181	122	2 mgm./day for 10 days	151	+29	6
203	159	2 mgm./day for 10 days	182	+23	6
151	108	2 mgm./day for 10 days	127	+19	7
152	110	2 mgm./day for 10 days	134	+24	
162	100	2 mgm./day for 10 days	132	+32	11
149	102	2 mgm./day for 10 days	122	+20	11
<i>168</i>	<i>120</i>		<i>145</i>	<i>+25</i>	<i>8</i>
		ADRENAL CORTICAL EXTRACT (i.p.)			
188	100	1.0 cc. daily for 6 days	112	+12	4
149	108	1.0 cc. daily for 6 days	132	+24	5
175	98	1.0 cc. daily for 6 days	116	+18	5
<i>171</i>	<i>102</i>		<i>120</i>	<i>+18</i>	<i>5</i>

(Explanatory notes follow table 3.)

From table 2 it may be seen that when the experiment was repeated on bilaterally adrenalectomized rats, the desoxycorticosterone gave a greater effect, but again there was only a partial restoration of the hypertension. The effects of the adrenal cortex extracts were inconstant and not marked.

When other steroids were substituted, no increases of blood pressure were noted (table 3). Sometimes the blood pressure was unstable during the injection

period and the fluctuations render the final value meaningless (as indicated by the question marks in column 5). Progesterone, administered in dosages of 2 mgm. daily for 10 days, produced no change of blood pressure in four animals and a definite decline in one. No toxic effects were noted. Testosterone produced a harmful effect, as evidenced by illness or weight loss in all of the rats and a decline in the blood pressure. When 0.17 mgm. of estradiol benzoate was given daily for only 6 days, there was no change of blood pressure during the injection period,

TABLE 2

The effect of desoxycorticosterone or of adrenal cortical extract on the blood pressure of hypertensive rats following bilateral adrenalectomy

BEFORE ADRE- NALECTOMY	AFTER ADRE- NALECTOMY	DESOXYCORTICOSTERONE IN OIL (I.M.)			
		Dose (3)	B.P. (4)	Change (5)	Period of decline (6)
					<i>days</i>
183	123	1 mgm./day for 6 days	182	+59	5
201	128	1 mgm./day for 6 days	177	+49	2
180	138	1 mgm./day for 6 days	166	+28	3
175	142	1 mgm./day for 6 days	156	+14	4
<i>185</i>	<i>133</i>		<i>170</i>	<i>+37</i>	<i>3.5</i>
		ADRENAL CORTICAL EXTRACT (I.P.)			
159	120	0.2 cc./day for 6 days	129	+9	6
161	128	0.2 cc./day for 6 days	144	+16	2
153	130	1.0 cc./day for 6 days	135	+5	1
168	138	1.0 cc./day for 6 days	164	+26	5
<i>160</i>	<i>129</i>		<i>143</i>	<i>+14</i>	<i>3.5</i>

(Explanatory notes follow table 3.)

but 5 out of the 6 animals died from 1 to 5 days after the last injection and the remaining animal became very ill.

DISCUSSION. The presence of functioning adrenal cortical tissue is absolutely essential for the maintenance of hypertension in both man and animals. It has already been pointed out above that when hypertension has been lost as the result of bilateral adrenalectomy, the hypertrophy of accessory islets of adrenal cortical tissue results in complete restoration of the hypertension, and similarly that when hypophysectomy is the cause, stimulation of the atrophied adrenal cortex with a purified adrenotrophic hormone produces the same result. Our failure to produce the same degree of restoration of the hypertension with the use of either desoxycorticosterone or with adrenal cortical extract suggests that the dosages used, which are ordinarily adequate for life maintenance and for the restoration of a normal electrolyte balance are inadequate for the maintenance of

TABLE 3

The effect of related steroids (sex hormones) on the blood pressure of hypophysectomized hypertensive rats

BEFORE HYPOPHYS- ECTOMY	AFTER HYPOPHYS- ECTOMY	PROGESTERONE IN OIL (I.M.)			
		Dose (3)	B.P. (4)	Change (5)	Period of decline (6)
					<i>days</i>
152	120	2 mgm./day for 10 days	121	0	0
152	118	2 mgm./day for 10 days	126	+8 (?)	0
155	125	2 mgm./day for 10 days	122	-3 (?)	0
151	128	2 mgm./day for 10 days	127	0	0
162	130	2 mgm./day for 10 days	105	-27	
<i>154</i>	<i>124</i>		<i>120</i>	<i>-4 (?)</i>	<i>0</i>
		TESTOSTERONE IN OIL (I.M.)			
181	120	2.5 mgm./day for 9 days	115	-5	Rat ill
149	121	2.5 mgm./day for 9 days	102	-19	Rat ill
150	121	2.5 mgm./day for 9 days	118	-3 (?)	Lost weight
175	119	2.5 mgm./day for 9 days	108	-11	Rat ill
<i>164</i>	<i>120</i>		<i>111</i>	<i>-9</i>	
		ESTRADIOL BENZOATE IN OIL (I.M.)			
161	118	0.17 mgm./day for 6 days	118	0	Died 1 day later
150	130	0.17 mgm./day for 6 days	130	0	Died 1 day later
180	130	0.17 mgm./day for 6 days	134	+4 (?)	Died 5 days later
170	118	0.17 mgm./day for 6 days	118	0	Died 3 days later
160	120	0.17 mgm./day for 6 days	120	0	Rat ill
153	118	0.17 mgm./day for 6 days	119	0	Died 5 days later
<i>162</i>	<i>121</i>		<i>123</i>	<i>0</i>	

(1) Average of 6 readings taken during the 2 weeks before hypophysectomy or adrenalectomy. The hypertension had been established 3-16 weeks before the ablation. Each line gives data for one animal.

(2) Average of 4 readings taken during the 2d or 3d week after hypophysectomy or adrenalectomy.

(3) Adrenal cortical extract (Upjohn) daily dosage was divided and given in 3 injections.

(4) Highest blood pressure at end of injection period.

(5) Column 4 minus column 2.

(6) Number of days after the last injection before blood pressure returned to pre-injection level (or, in the estradiol group, before death).

Figures in italics indicate mean values for each group.

a hypertension. The alternative hypothesis has not been ruled out, namely, that there is an additional blood pressure raising factor elaborated by the intact adrenal cortex which is not present in the usual extracts.

Even though estradiol, testosterone or progesterone may show some of the properties of adrenal cortical steroids, it is clear that no one of them is capable of raising the blood pressure in the presence of adrenal cortical insufficiency.

Leathem and Drill (14) noted that diethylstilbestrol had a hypotensive and lethal effect upon hypophysectomized rats, but not upon normal rats. Our results indicate that both estradiol and testosterone also have a toxic or lethal effect after hypophysectomy.

CONCLUSIONS

1. Following the establishment of experimental renal hypertension in rats, hypophysectomy or adrenalectomy causes a fall of blood pressure which may in some cases reach levels below normal. The hypertension may then be partially, though rarely completely restored by the administration of desoxycorticosterone or adrenal cortical extracts.

2. Progesterone, testosterone or estradiol have no blood pressure raising properties in these animals.

3. Testosterone and estradiol exhibit toxic or lethal effects following hypophysectomy.

4. Either larger amounts of desoxycorticosterone or adrenal cortical extract are required for the maintenance of hypertension than for life or for normal electrolyte balance, or there is an additional unknown factor from the adrenal cortex which is concerned with the maintenance of blood pressure.

REFERENCES

- (1) OGDEN, E., E. W. PAGE AND E. ANDERSON. *This Journal* **141**: 389, 1944.
- (2) ANDERSON, E., E. W. PAGE, C. H. LI AND E. OGDEN. *This Journal* **141**: 393, 1944.
- (3) GOLDBLATT, H. *Ann. Int. Med.* **11**: 69, 1937.
- (4) PAGE, I. H. *This Journal* **122**: 352, 1938.
- (5) COLLINS, D. A. AND E. H. WOOD. *This Journal* **123**: 224, 1938.
- (6) HOUSSAY, B. A. AND L. DEXTER. *Ann. Int. Med.* **17**: 451, 1942.
- (7) GAUDINO, N. M. *Rev. Soc. Argent. de Biol.* **20**: 529, 1944.
- (8) COLLINGS, W. D., E. OGDEN AND A. N. TAYLOR. *Fed. Proc.* **5** (Part II): 19, 1946.
- (9) PERERA, G. A. *J. A. M. A.* **129**: 537, 1945.
- (10) LEATHEM, J. H. AND V. A. DRILL. *Endocrinology* **35**: 112, 1944.
- (11) DELL'ORO, R. *Rev. Soc. Argent. de Biol.* **18**: 13, 1942.
- (12) INGLE, D. J. *Ann. Rev. Physiol.* **31**: 419, 1942.
- (13) WILLIAMS, J. R., T. R. HARRISON AND A. J. GROLLMAN. *J. Clin. Investigation* **18**: 373, 1939.
- (14) LEATHEM, J. H. AND V. A. DRILL. *This Journal* **139**: 17, 1943.

THE ELECTROCARDIOGRAM OF RATS ON VITAMIN E DEFICIENCY

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Following the report of the effect of vitamin E deficiency on cross striated musculature of rats by Evans, Emerson and Telford (1), the author became interested in its possible effects on cardiac muscle. Olcott (2) reported that heart muscle is not affected histologically in rats 23 to 36 days old, born of E deficient mothers. S. Americano Freire (3) reported lesions in the myocardium of rats identical with those found in the skeletal muscles. The rats, born of mothers maintained on an E low diet during pregnancy, were kept on the E low diet for 260 to 340 days and then sacrificed for histological study. Mackenzie (4) reported the absence of myocardial lesions in the rabbit after several attacks and cures of muscular dystrophy over a period of 16 to 32 weeks. He did report the finding in the ventricles of several accumulations of cells with large, pale, elliptical or irregular nuclei. Mason (5) reported that the heart, lung and spleen of rats on an E low diet contain more vitamin E than other organs including body fat, and that these same organs receive more vitamin E than other tissues, except the liver which acts as a storehouse, when the rats are kept on E high diet. Houchin and Smith (6) reported that the hearts of young rabbits on E deficient diets were more susceptible to posterior pituitary extract and had a higher resistance to cardiac glycosides than the hearts of normal young rabbits. They also reported a probable cardiac dilatation as revealed by x-ray. Recently, Telford, Swegart and Schoene (7) reported blood pressure studies on E deficient rats. These rats showed a reduction of 29.4 per cent in arterial pressure over the normal control animals during a period of one year. There was no essential increase in the per cent difference at the end of two years. Histological examination of the vascular system failed to show any particular lesion that might explain the reduction.

In view of our obviously incomplete knowledge of the significance of vitamin E in cardiac physiology, it was thought that it might be worth while to study the course of a series of electrocardiograms taken on rats maintained on a vitamin E low diet for a period of one year.

METHOD. Five groups of four rats each were placed at weaning (21 days) on Olcott's diet (2). Three of the groups were males and two groups were females. One member of each group served as a control and was given each day of the week except Sundays a supplement of alpha-tocopherol in ethyl laurate. To ensure a more than adequate intake of alpha-tocopherol for the controls, a level of 3 mgm. per kilogram of body weight was selected. This

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level was decided upon after studying the reports of Evans, Emerson and Emerson (8) and of Evans and Emerson (9). The alpha-tocopherol² was dissolved in ethyl laurate and given daily except Sundays with a small orifice medicine dropper. The solution was made up fresh every two weeks, evacuated and kept in a refrigerator.

A Sanborn Electrocardiograph was used with a camera speed of 5 cm. per second which is sufficient to read accurately small time intervals. A standard of 1 mv. = 1 cm. was used in order to have as high frequency as possible for the string. The standardization curve showed that 0.006 sec. was required for the shadow of the string to move one centimeter. Since the QRS complex is completed in 0.01 second in young rats, the sensitivity of the apparatus was probably insufficient to record faithfully the rapid changes of the QRS complex. However, in a series of electrocardiograms taken on the same animal over a period of a few days, each record was identical, and it was felt that the apparatus would show any changes that resulted from the diet.

Tapes were attached to the four extremities and the rat was gently stretched, abdomen down, on a cork board and held there by pins passed through the tapes into the board. A two-fingered clamp on the neck kept the rat from turning its head and nibbling on the electrodes. After a little handling and a few trials the rat would remain fairly quiet while the electrocardiogram was being made. The electrodes were made of pure sheet zinc obtained from old-fashioned electrocardiograph electrodes and covered with a tape of soft material sufficiently long to wrap twice around the limb of the rat. The pieces of zinc were cut in the form of a T. The cross-bar of the T was made 1 cm. wide and 2 cm. long. The leg of the T was attached to the leads of the electrocardiograph by short flexible wires. Before use the electrodes were soaked in saline solution and dipped into a soft paste made of starch and salt. The paste was made by dissolving 38 g. sodium chloride in 100 ml. of water, bringing to a boil and adding 3.5 g. potato starch suspended in the least amount of water possible. Before applying the electrodes, the legs of the animals were washed with soap and water to remove the oily substance in the fur and ensure a good contact. The electrodes were then applied and bent to fit tightly against the curvature of the leg. The tape was wrapped twice around the electrode and leg and held with a wood clip shaped very much like a tiny clothes-pin but using an elastic band instead of a metal spring.

Electrocardiograms were taken at 4 week intervals for the first 24 weeks and then at 8 week intervals.

RESULTS. Heart rate at the age of 4 weeks, 1 week after weaning, averaged 540 varying individually from 430 to 600, but most fell in a group between 500 and 550. The heart rate fell steadily with increasing age and the variations became less extreme until at the age of 1 year the average rate became 405 per min. There was no difference between the controls and the E deficient rats.

² The alpha-tocopherol was obtained from Eastman Kodak Company Research Laboratories.

The P-R interval was 0.04 sec. at the age of 4 weeks and in most cases had lengthened to 0.045 sec. at the age of 1 year. There was no difference between the controls and the E deficient rats.

The QRS was 0.01 sec. at the age of 4 weeks and lengthened to 0.015 sec. at the age of 1 year. In 5 of the 15 E deficient rats the QRS lengthened to 0.018 sec., but in the other 10 there was no difference from the controls.

The T wave varied in length from 0.02 to 0.05 sec. in lead I and from 0.06 to 0.09 in leads II and III. Very rarely was there any interval between the completion of the QRS complex and the beginning of the T wave.

The P wave always occurred on the descending limb of the previous T wave except in the occasional case in which the heart rate was below 400 per min.

The R wave varied from 5.0 to 7.5 mv. in lead II and from 2.5 mv. to 5.5 mv. in lead I. Lead III was intermediate. The variation was less in the individual rats. There was no difference between the controls and the E deficient rats.

DISCUSSION. The commercial electrocardiograph is probably insufficiently sensitive to record accurately the rapid variations of the QRS complex of small animals. A short, tense string with an amplifier such as described by Rappaport and Rappaport (10) or by Hundley, Ashburn and Sebrell (11) should give more faithful records. As electrocardiograms taken with a commercial instrument on the same rat over a period of several days were identical and yet different from those taken on other rats, it is thought that the instrument is sufficiently sensitive to indicate changes brought about by experimental means. The method described in this paper allows one individual alone to do the work.

The only difference between the electrocardiograms reported in this paper and the normals reported by Hundley, Ashburn and Sebrell (11) is the length of the QRS interval. They report a time interval of 0.006 to 0.013 sec., whereas the intervals reported here are 0.01 to 0.015 sec. It is possible that the amplifier increased the sensitivity of the instrument in their experiments; however, they used a standard of 1 mv. = 2 cm. which decreases the frequency of the string.

Since it was shown by Evans and Emerson (9) that as little as 0.1 mgm. of alpha-tocopherol daily would protect against sterility up to about 4½ months and is adequate for normal growth and normality of striated musculature, it was believed that this observation might provide a test of the effect of the diet. Several of the male rats, after being on the E low diet for 100 days, were placed in a cage with several normal female breeding rats for a period of 3 weeks. The male rats showed no interest in the females, nor did any pregnancies result.

CONCLUSIONS

The electrocardiogram of rats maintained for one year on an E deficient diet is not different from the electrocardiogram of normal rats with the possible exception of a slight widening of the QRS complex that occurred in 5 out of 15 test rats.

REFERENCES

- (1) EVANS, H. M., G. A. EMERSON AND I. R. TELFORD. *Proc. Soc. Exper. Biol. and Med.* **38**: 625, 1938.

- (2) OLCOTT, H. S. *J. Nutrition* **15**: 221, 1938.
- (3) FREIRE, S. A. *Brasil-Medico* **55**: 308, 1941.
- (4) MACKENZIE, C. G. *Proc. Soc. Exper. Biol. and Med.* **49**: 313, 1942.
- (5) MASON, K. E. *J. Nutrition* **23**: 71, 1942.
- (6) HOUCHIN, O. B. AND P. W. SMITH. *This Journal* **141**: 242, 1944.
- (7) TELFORD, I. R., J. E. SWEGERT AND F. C. SCHOENE. *This Journal* **143**: 214, 1945.
- (8) EVANS, H. M., G. A. EMERSON AND O. H. EMERSON. *Anat. Rec.* **74**: 257, 1939.
- (9) EVANS, H. M. AND G. A. EMERSON. *J. Nutrition* **26**: 555, 1943.
- (10) RAPPAPORT, M. B. AND I. RAPPAPORT. *Am. Heart J.* **26**: 662, 1943.
- (11) HUNDLEY, J. M., L. L. ASHBURN AND W. H. SEBRELL. *This Journal* **144**: 404, 1945.

FACTORS GOVERNING THE RATE OF EXCRETION OF TITRATABLE ACID IN THE DOG¹

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The capacity of the kidney to excrete a part of the buffer acid produced in the metabolism of fats and proteins in free titratable form enables the body to conserve its limited stores of fixed base. Phosphoric, uric, beta-hydroxybutyric and acetoacetic acids bind nearly their full equivalence of base in the alkaline body fluids and glomerular filtrate. As the filtrate proceeds through the distal segments of the renal tubules, it is converted into acid urine (6). This change in reaction is effected by the exchange of hydrogen ions formed within the tubular cells for ions of fixed base in the tubular urine (9). The base thus salvaged is returned to the renal venous blood as bicarbonate; the hydrogen ions and buffer anions are eliminated in the urine as titratable acid. The present paper is concerned with the definition and elucidation of the factors which determine the rate at which titratable acid is eliminated, i.e., the factors which determine the rate of exchange of hydrogen ions for ions of fixed base in the renal tubules. It has been found that three major factors are concerned: 1, the buffer content of the tubular urine; 2, the strength of the buffer acid excreted; and 3, the degree of acidosis, i.e., the extent of the depletion of the body stores of available base.

METHODS. The data presented in this paper were obtained in 18 experiments performed on four normal female mongrel dogs. Experiments 1 through 14 were performed on dogs 1 and 2, which had been shown in previous studies to have closely similar renal functional capacities. Urine was collected by catheter and the bladder was washed with distilled water at the end of each clearance period. Since the urines for the most part were acid and highly buffered, loss of carbon dioxide introduced no significant error in the determination of pH and titratable acid (2, 5). In the majority of the experiments blood samples were drawn from the jugular vein, in others from the femoral artery. Creatinine and phosphate were administered in infusions pumped into the saphenous vein at a constant rate. Acidosis was induced by the oral administration of ammonium chloride or by the intravenous infusion of hydrochloric acid. The chemical methods employed and the calculations made have been described in previous communications (8, 9).

RESULTS. *The relationship between the rate of buffer excretion and the rate of excretion of titratable acid.* Henderson (3) has pointed out that the titratable acid of normal human urine is largely monobasic phosphate, and that the quan-

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tity of acid eliminated is roughly proportional to the quantity of phosphate excreted, other conditions remaining constant. Experiment 1 in table 1 inquires in a systematic fashion into this relationship in the acidotic dog. Acido-

TABLE 1

Experiments on an acidotic dog which illustrate the relationship between the rate of excretion of titratable acid and the rate of excretion of buffer

In experiment 1, the major urinary buffer was phosphate; in experiment 2, creatinine.

TOTAL CON- CUR- RENT TIME	PLASMA CONCENTRATION			GLOMERULAR FIL- TRATION RATE	URINE		PHOSPHATE			CREATININE EXCRETED	TITRATABLE ACIDITY OF URINE				
	Phos- phate	Crea- tinine	CO ₂ com- bining power		Flow	pH	Filtered	Excreted	Reabsorbed		Obs- erved	Calculated from			
												Phos- phate ex- creted	Crea- tinine ex- creted	Total buffer excreted	
min.	mM./ L.	mgm. %	vol. %	cc./ min.	cc./ min.	mM./ min.	mM./ min.	mM./ min.	mM./ min.	mEq./ min.	mEq./ min.	mEq./ min.	mEq./ min.	% of Ob- served	
Experiment 1; dog 2															
75- 85	2.87	23.4	19.4	68.0	4.9	5.57	0.195	0.081	0.114	0.141	0.087	0.059	0.028	0.087	100.0
85- 95	3.03	23.0	19.2	71.4	7.3	5.66	0.216	0.119	0.097	0.145	0.114	0.085	0.024	0.109	95.6
110-120	4.13	23.0	20.4	68.4	8.6	5.29	0.275	0.180	0.095	0.139	0.181	0.135	0.045	0.180	99.4
120-130	4.26	21.8	19.9	69.0	8.9	5.36	0.294	0.191	0.103	0.134	0.181	0.143	0.038	0.181	100.0
145-155	5.64	22.4	19.8	63.4	8.6	5.58	0.357	0.259	0.098	0.126	0.217	0.189	0.024	0.213	98.1
155-165	6.46	22.9	20.4	62.8	9.0	5.73	0.405	0.308	0.097	0.127	0.239	0.216	0.018	0.234	97.9
180-190	9.67	25.0	22.5	61.0	10.1	6.08	0.595	0.469	0.126	0.135	0.308	0.283	0.009	0.292	95.0
190-200	10.9	25.9	23.5	60.2	10.6	6.29	0.656	0.544	0.112	0.138	0.309	0.296	0.006	0.302	97.8
215-225	14.1	26.2	26.8	60.9	12.0	6.33	0.859	0.736	0.123	0.142	0.386	0.387	0.005	0.392	101.5
225-235	15.9	27.2	28.0	61.0	13.1	6.39	0.970	0.862	0.108	0.150	0.431	0.431	0.005	0.436	101.2
Experiment 2; dog 2															
65-75	1.42	21.8	24.8	66.8	1.4	5.12	0.095	0.024	0.071	0.129	0.078	0.019	0.054	0.073	93.6
75- 85	1.38	20.3	24.8	71.2	1.6	5.10	0.098	0.028	0.070	0.128	0.079	0.022	0.055	0.077	97.4
100-110	1.21	32.6	25.0	69.3	3.2	5.27	0.084	0.019	0.065	0.200	0.085	0.015	0.066	0.081	95.4
110-120	1.21	36.9	24.8	69.4	3.4	5.27	0.084	0.019	0.065	0.227	0.095	0.015	0.076	0.091	95.9
135-145	1.30	66.3	22.8	65.6	5.1	5.44	0.085	0.024	0.061	0.385	0.119	0.019	0.096	0.115	96.6
145-155	1.28	70.2	22.7	68.8	5.3	5.48	0.088	0.024	0.064	0.427	0.121	0.019	0.098	0.117	96.6
170-180	1.24	135	23.8	71.0	6.7	5.81	0.088	0.022	0.066	0.848	0.124	0.018	0.103	0.121	97.6
180-190	1.19	149	25.2	68.6	6.0	5.88	0.082	0.019	0.063	0.905	0.114	0.014	0.096	0.110	96.4
205-215	1.10	232	25.7	70.2	6.7	6.02	0.077	0.014	0.063	1.44	0.125	0.010	0.112	0.122	97.5
215-225	1.10	257	26.8	69.3	7.3	6.10	0.076	0.011	0.065	1.48	0.114	0.008	0.104	0.112	98.2

sis was induced by the oral administration of 6 grams of ammonium chloride per day for a week preceding the experiment. To avoid acute changes in acid base balance no ammonium chloride was administered on the day of the experiment. Carbon dioxide combining powers of 19 volumes per cent in the initial experimental periods are indicative of a severe acidosis. Phosphate was infused at progressively increasing rates to raise the plasma concentration to levels from

3 to 15 times the normal value of 1 millimol per liter. Since the quantity of phosphate reabsorbed per unit of time remained essentially constant throughout the experiment, the quantity excreted increased in direct proportion to the increase in plasma concentration. It is apparent that the titratable acidity of the urine varied as a function of the quantity of phosphate excreted. The maximum rate of excretion of titratable acid attained in this experiment, namely, 0.431 milliequivalent per minute, is equivalent to the excretion of 6,100 cc. of N/10 acid per day, a value far in excess of the maximum rate observed in the

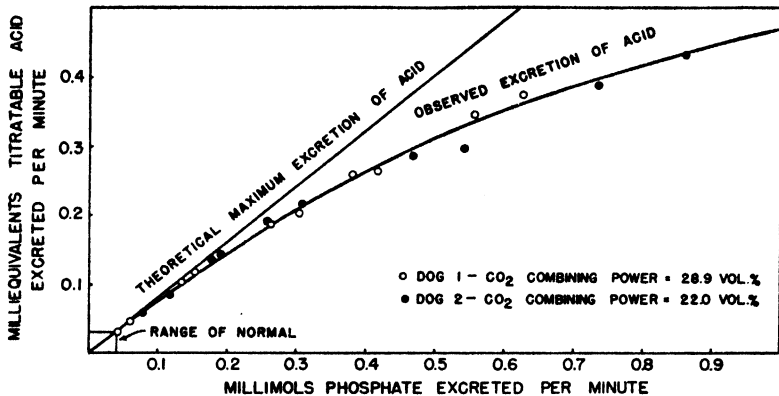


Fig. 1. The relation between the rate of excretion of titratable acid and the rate of excretion of phosphate in the acidotic dog.

human even in severe diabetic ketosis. The factor responsible for this high rate of excretion of acid is the high rate of excretion of phosphate. During the last 4 clearance periods in which the rate of acid elimination was high, the carbon dioxide combining power of the plasma increased from 22.5 to 28.0 volumes per cent. It is evident that the kidney can effect a rapid restoration of the alkali reserve of the plasma when it is provided with a large excess of phosphate.

The values for observed titratable acidity in table 1 were obtained by titrating the urines electrometrically to the pH of the plasma.² This titration measures the total acid elimination due to all urinary buffers and thus includes not only phosphate but creatinine as well. In measuring glomerular filtration rate considerable quantities of creatinine were administered (note plasma concentrations of 21.8 to 27.2 mgm. per cent). The individual contributions of phosphate and creatinine to the titratable acidity of the urine were calculated from their rates of excretion, pK' values, and the urine pH, using the Henderson-Hasselbalch equation. The calculated values, listed in the columns on the right of table 1, are in close agreement with the observed values. That moiety of the calculated titratable acid contributed by phosphate is plotted in figure 1 against the rate of phosphate excretion. Identical experiments on two dogs of similar

² A pH of 7.35 was arbitrarily chosen as the end point for the titration. In comparable experiments in which arterial pH was determined directly, values from 7.25 to 7.35 were observed.

renal functional capacity and in comparable states of acidosis are included. The correlation between the rate of acid excretion and the rate of phosphate excretion is evident. The line labelled *theoretical maximum excretion of acid* is that quantity which would have been eliminated if in each instance urine of maximal acidity (pH 4.8) had been formed. The deviation from the theoretical maximum rate is greater the higher the rate of phosphate excretion. The basis for this deviation is evident in the figures of table 1. As the rate of excretion of phosphate and titratable acid increased, urine pH likewise increased. Thus, although more titratable acid was eliminated at the higher rates of phosphate excretion, the buffer potentialities of the phosphate were less completely utilized.

The relationship between the strength of the buffer acid and rate of excretion of titratable acid. One would predict that the stronger the buffer acid the less readily would the renal tubules exchange hydrogen ions for ions of fixed base bound by that buffer in the tubular urine. Thus phosphate with a pK' of 6.8 yields more than 80 per cent of its secondary base in urine of pH 6.0. The concentration of ionized hydrogen in such urine is only 20 times that of the plasma. On the other hand beta-hydroxybutyric acid with a pK' of 4.7 yields less than 50 per cent of its base in urine of maximum acidity, namely, urine of pH 4.8 (4). The concentration of ionized hydrogen in such urine is 400 times that of the plasma. Obviously the renal work involved in acidifying the urine is less the lower the concentration gradient against which hydrogen ions must be transferred. Thus phosphate is a more effective urinary buffer than is beta-hydroxybutyric acid, and it in turn is more effective than the somewhat stronger acetoacetic acid.

Creatinine in acid urine is a buffer comparable in strength to beta-hydroxybutyric acid for its acid pK' is 4.97. Because of the availability of creatinine and the simplicity of its analysis we have chosen to compare the relative effectiveness of creatinine and phosphate as urinary buffers rather than beta-hydroxybutyrate and phosphate. It is evident from experiment 2 in table 1 that the rate of excretion of titratable acid is correlated with the rate of excretion of creatinine. In this experiment creatinine was administered in increasing amounts to raise the plasma concentration from 20 to 250 mgm. per cent. The rate of excretion of creatinine rose from 0.129 millimol per minute to 1.48 millimols per minute and the observed excretion of titratable acid from 0.078 milliequivalent per minute to 0.125 milliequivalent per minute. Although no phosphate was administered the normal spontaneous excretion of this buffer was sufficient to account for a significant fraction of the observed titratable acid. The individual contributions of the two buffers as calculated from their rates of excretion, pK' values and urine pH are listed on the right of table 1. It is apparent that the creatinine moiety of the titratable acid increased as the excretion of creatinine increased.

A similar experiment was performed on dog 1 in a comparable state of acidosis, and the results of the two experiments are plotted in figure 2. The data obtained in like experiments with phosphate are included to facilitate comparison. It is apparent from this graph that phosphate is a better urinary buffer

than is creatinine, and that when equimolar quantities of the two buffers are presented to the kidney, the excretion of acid is much greater with phosphate than with creatinine. Thus the stronger the buffer acid the less effectively does

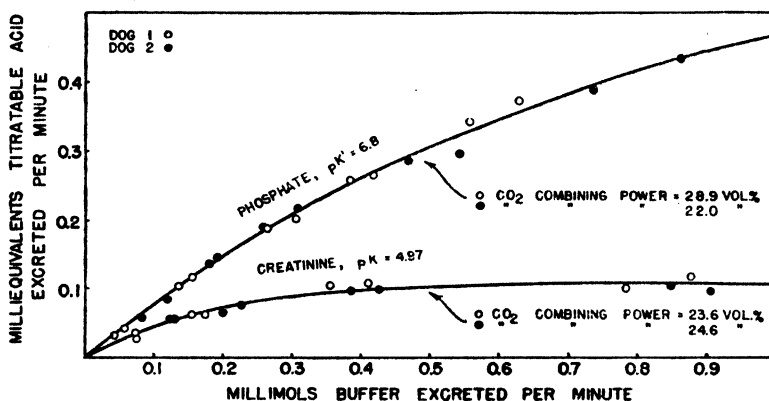


Fig. 2. A comparison of the effectiveness of phosphate and of creatinine as urinary buffers in the acidotic dog.

TABLE 2

An experiment on an acidotic dog which illustrates the greater effectiveness of phosphate than of creatinine as a urinary buffer

TOTAL CON- CURRENT TIME	PLASMA CONCENTRATION			GLOMERULAR FIL- TRATION RATE	URINE		PHOSPHATE			CREATININE EXCRETED	TITRATABLE ACIDITY OF URINE				
	Phos- phate	Crea- tinine	CO ₂ com- bining power		Flow	pH	Filtered	Excreted	Reabsorbed		Ob- served	Calculated from			
												Phos- phate ex- creted	Crea- tinine ex- creted	Total buffer excreted	
min.	mM./ L.	mgm. %	vol. %	cc./ min.	cc./ min.		mM./ min.	mM./ min.	mM./ min.	mM./ min.	mEq./ min.	mEq./ min.	mEq./ min.	mEq./ min.	% of Ob- served
Experiment 9; dog 1															
90-100	0.642	118	39.3	88.5	5.2	6.13	0.057	0.001	0.056	0.924	0.057	0.001	0.057	0.058	101.8
100-110	0.619	117	38.8	93.3	8.6	6.19	0.058	0.003	0.055	0.964	0.052	0.002	0.052	0.054	104.0
110-120	0.619	119	38.0	95.1	11.2	6.15	0.059	0.004	0.055	1.000	0.060	0.003	0.060	0.063	105.0
								0.003		0.963	0.056	0.002	0.056		
140-150	12.1	136	39.7	80.9	14.6	6.50	0.979	0.890	0.089	0.971	0.424	0.397	0.025	0.422	99.5
150-160	13.6	143	40.8	80.5	15.7	6.59	1.095	1.003	0.095	1.016	0.419	0.400	0.020	0.420	100.2
160-170	14.9	148	41.2	79.1	16.9	6.67	1.180	1.102	0.080	1.034	0.411	0.385	0.017	0.402	98.0
								0.998		1.007	0.418	0.394	0.021		

the kidney exchange hydrogen ions for the ions of fixed base bound by that buffer in the tubular urine.

Experiment 9 in table 2 illustrates in a more direct fashion the greater effectiveness of phosphate than of creatinine as a urinary buffer. The plasma concentration of creatinine was maintained at a level sufficiently high to cause the

excretion of approximately one millimol of creatinine per minute throughout the experiment. In the initial three clearance periods 0.056 milliequivalent per minute of titratable acid was eliminated, practically all as a consequence of the buffering power of creatinine. Phosphate was then infused at such a rate as to cause the excretion of one millimol of this buffer per minute. The excretion of titratable acid rose to 0.418 milliequivalent per minute, the vast majority of which was contributed by phosphate, i.e., 0.394 milliequivalent by phosphate as opposed to 0.021 milliequivalent by creatinine.

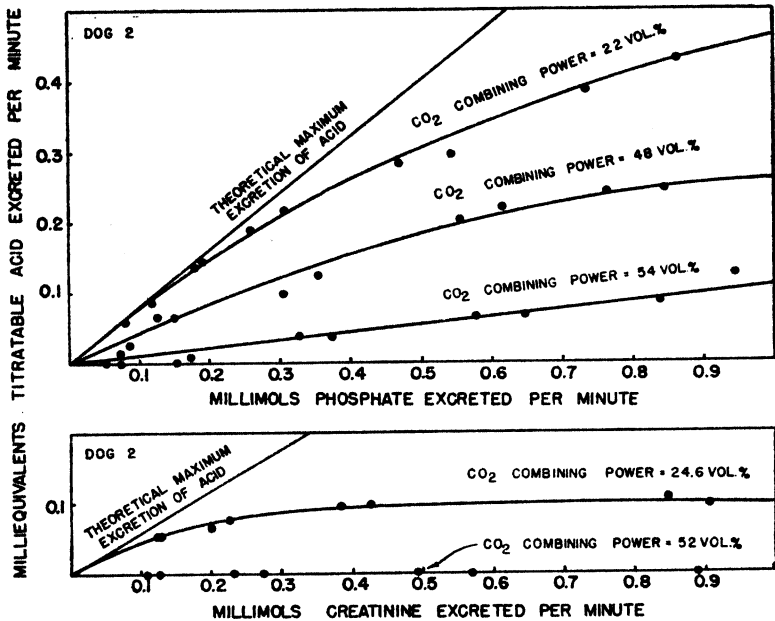


Fig. 3. The relation between the rate of excretion of titratable acid and the degree of acidosis at a series of rates of excretion of buffer. In the upper graph the major urinary buffer was phosphate; in the lower graph, creatinine.

The relationship between the degree of acidosis and the rate of excretion of titratable acid. It has been observed previously (1, 7) that the rate of excretion of titratable acid is closely correlated with the acid load of the body, thus with the need of the body for the conservation of base. For the most part these observations have been complicated by the fact that increased acid load and increased buffer excretion are commonly associated. To dissociate these two factors experiments similar to those summarized in table 1 were performed with the animal in differing states of acidosis. In the upper half of figure 3 are presented the data obtained in three experiments in which phosphate was infused. The states of acidosis are characterized by the carbon dioxide combining powers noted on the experimental curves, a combining power of 22 volumes per cent indicating severe acidosis, one of 54 volumes per cent falling within the range of normal. It is apparent that at any one of a series of rates of excretion of buffer

the more severe the acidosis the greater is the excretion of titratable acid. Furthermore, the more severe the acidosis the more nearly does the kidney approach its maximum theoretical capacity for the excretion of acid. It is interesting to note in animals in which the carbon dioxide combining power is within the normal range that the infusion of phosphate increases to a very significant degree the excretion of titratable acid. Thus the animal normally may be said to be in a state of mild acidosis.

In the lower half of figure 3 are presented two experiments in which the effects of creatinine administration on titratable acid elimination were compared in the normal and in the acidotic state. Results with this buffer confirm in general the thesis that the lower the alkali reserve of the body and the greater the need for base conservation, the greater is the excretion of titratable acid.

The tubular mechanism of exchange of hydrogen ions for ions of fixed base is to all intents immediately and reversibly sensitive to alterations in the alkali reserve. This fact is demonstrated by experiments presented in table 3. In these two experiments phosphate was infused at such a rate as to cause the excretion of about 0.6 millimol of this buffer per minute. The initial two control periods of experiment 17 were performed with the animal in a state of normal acid base balance as indicated by the arterial plasma bicarbonate concentration of 21 millimols per liter, pH of 7.38 and $p\text{CO}_2$ of 36 mm. Hg. The excretion of titratable acid amounted on an average to 0.080 milliequivalent per minute. One-tenth normal hydrochloric acid was then infused intravenously for 50 minutes at a rate of 10 cc. per minute, during which time the plasma bicarbonate concentration fell to 9.9 millimols per liter. The rate of excretion of titratable acid increased as the plasma bicarbonate fell, reaching a final value of 0.300 milliequivalent per minute. Preceding experiment 18, ammonium chloride was administered for a period of one week to lower chronically the plasma bicarbonate concentration to essentially the same level as that attained acutely at the end of experiment 17. Titratable acid elimination in the two control periods was roughly the same as that at the end of the preceding experiment. Sodium bicarbonate was then infused at such a rate as to elevate the plasma bicarbonate to the normal range within a period of 50 minutes. The excretion of titratable acid declined progressively and fell to a value which approximated that of the control periods of experiment 17. It is evident that if any lag exists in the response of the mechanism of titratable acid excretion to changes in acid base balance, it is a very insignificant one.

DISCUSSION. The interrelationships of the pH of the urine and the rates of excretion of titratable acid and phosphate are presented in figure 4. The 3 smooth curves are titration curves of 0.2, 0.5 and 1.0 millimol of phosphate over the physiological range of urine pH. Under conditions such that the pH of the glomerular filtrate is 7.4 and 1 millimol of phosphate is excreted per minute, the exchange of hydrogen ions for ions of fixed base in the distal segments of the renal tubules would effectively titrate the tubular urine along the 1 millimol curve downward and to the left. If the final urine were of pH 6.6, 0.400 milliequivalent of titratable acid would be excreted per minute. The

concentration of hydrogen ions in this urine would be only 6 times that of the plasma, and the gradient across the tubules against which the hydrogen ion transfer is effected would be small. If only 0.5 millimol of phosphate were excreted, the tubular titration would have to proceed to pH 4.8 in order that 0.400 milliequivalent of titratable acid could be excreted. At this pH the concentration of hydrogen ions in the urine would be 400 times that of the plasma and the gradient opposing the transfer very high. If 0.2 millimol of phosphate were excreted at pH 4.8, only 0.16 milliequivalent of titratable acid could possibly be formed, and this quantity only against the high gradient of 400 to 1.

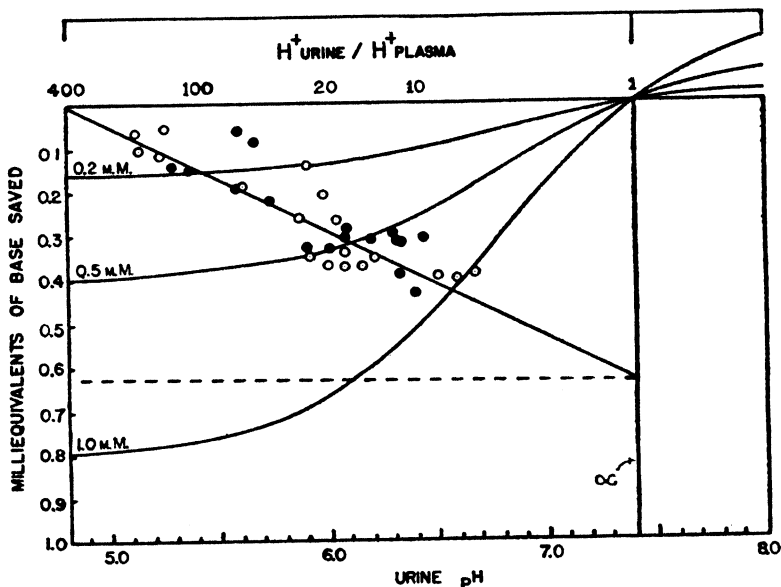


Fig. 4. A diagrammatic representation of the interrelations of urine pH, rate of phosphate excretion and rate of base conservation in the acidotic dog. For details see text.

The points on the graph are data obtained on dogs 1 and 2 during the infusion of varying quantities of phosphate but under more or less standard conditions of acidosis, namely, carbon dioxide combining powers of 20 to 30 volumes per cent. The rate of excretion of the titratable acid contributed by phosphate is plotted against urine pH. Urine samples represented by the points in the upper left hand section of the graph, i.e., highly acid urines of low titratable acidity, contained relatively small quantities of phosphate. Urine samples represented by points lower and to the right, i.e., less acid urines of high titratable acidity, contained large quantities of phosphate. In effect this graph is a triaxial plot of titratable acid, pH and phosphate excretion, utilizing the titration curves as a third scale of phosphate excretion. If urine contains little buffer the transfer of small quantities of hydrogen ions establishes a high gradient between urine and plasma, effectively limiting further transfer, and the rate of excretion of titratable acid is negligible. If the urine contains much buffer, the transfer of

even large quantities of hydrogen ions establishes only a low gradient. There would appear therefore to be an inverse relationship between the quantity of hydrogen ions which the tubules can transfer and the gradient against which that transfer must be effected. The vertical line at pH 7.4 represents the titration curve of an infinite quantity of phosphate. Were infinite amounts of phosphate excreted, the rate of elimination of titratable acid would approach 0.625 milliequivalent per minute under the conditions of acidosis prevailing in these experiments. The slope of the diagonal line and its intercept on the line of infinite excretion would vary as a function of the degree of acidosis, i.e., the more

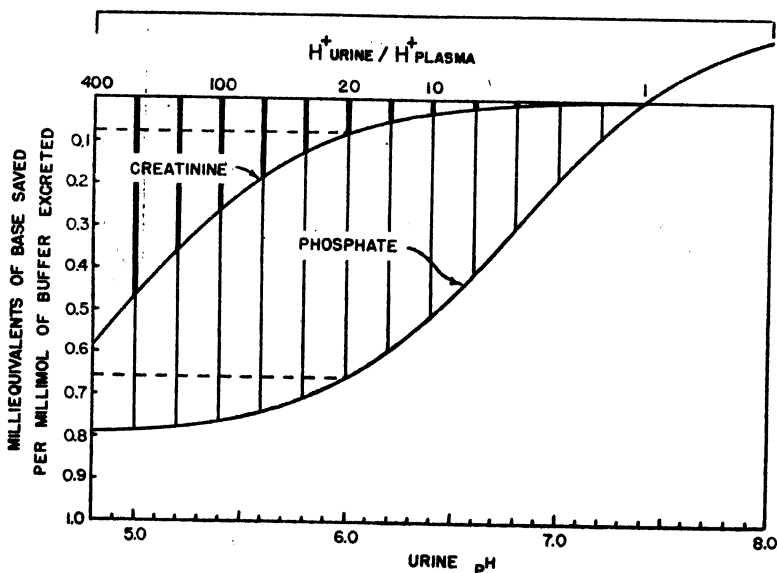


Fig. 5. A diagrammatic representation of the interrelations of urine pH, base conservation, and nature of the buffer excreted in the acidotic dog. For details see text.

severe the acidosis the greater would be the slope of the line and the greater the elimination of titratable acid.

The reason for the greater effectiveness of phosphate than of creatinine or other buffers of comparable strength in the renal processes involved in the excretion of acid is evident in the titration curves of figure 5. The upper curve is the titration curve of 1 millimol of creatinine, the lower that of 1 millimol of phosphate. In forming urine of pH 6.0 hydrogen ions are transferred against the relatively low gradient of 20 to 1. If the urinary buffer is phosphate some 0.660 milliequivalent of hydrogen ion per millimol of phosphate excreted could be transferred before this gradient is attained. If the urinary buffer is creatinine only 0.080 milliequivalent per millimol could be transferred before this gradient is attained. The excretion of 0.660 milliequivalent of acid per millimol of creatinine excreted would require the establishment of a concentration gradient well over 400 to 1, a gradient in excess of that which the kidneys can attain.

Since beta-hydroxybutyric and acetoacetic acids are even stronger acids than creatinine, their deficiencies as urinary buffers would be even more exaggerated. Hence in severe diabetic ketosis, the elimination of large quantities of these acids necessitates a considerable loss of base.

The correlation between the rate of excretion of titratable acid and the extent of the depletion of the body reserves of available base is a direct one, as is evident from experiments presented in figure 3 and table 3. A possible means by which the exchange of hydrogen ions for ions of fixed base could be regulated by the plasma bicarbonate concentration is illustrated by the diagrams of figure 6. It is known that bicarbonate is reabsorbed in the proximal and distal tubules by

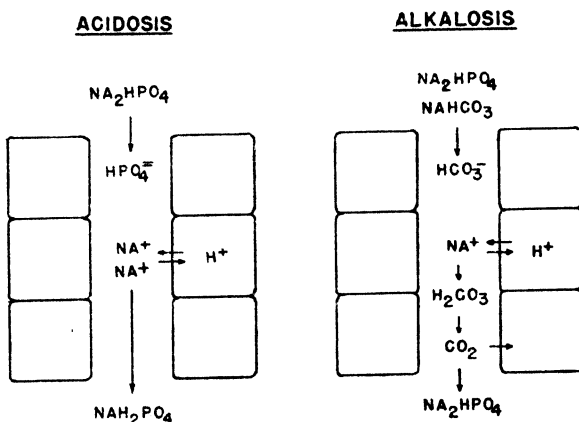


Fig. 6. A diagrammatic explanation of the observed correlation between the rate of excretion of titratable acid and the extent of the depletion of the alkali reserve. The two diagrams illustrate that segment of the distal renal tubule which is concerned with acidification of the urine. For details see text.

mechanisms of distinctly different functional properties. Thus proximal reabsorption is carried out isohydrically, distal reabsorption anisohydrically (6). The distal reabsorptive mechanism for bicarbonate appears to be the same as that responsible for the exchange of hydrogen ions for ions of fixed base (10). In conditions of acidosis in which the quantity of bicarbonate filtered is small, the major portion would be reabsorbed in the proximal segments of the tubules and relatively little would reach the site of urine acidification (cf. left hand diagram of fig. 6). Therefore hydrogen ions would be exchanged for the ions of fixed base bound by urinary buffers and titratable acid would appear in the urine. When the plasma concentration of bicarbonate is within or above the range of normal, bicarbonate would enter the distal tubules in considerable quantities (cf. right hand diagram of fig. 6). The exchange of hydrogen ions for base would now form carbonic acid, and being unstable this acid would break down to carbon dioxide and water. Because of the competition between buffer and bicarbonate in supplying base, relatively less buffer would be transformed into titratable acid. According to this concept the quantity of bicarbonate

which enters the distal tubule is an important factor in determining the rate of formation of titratable acid. Since this quantity is proportional to the plasma concentration of bicarbonate, acid excretion is correlated with the extent of the depletion of the body reserves of available base.

CONCLUSIONS

Three major factors determine the rate at which the normal kidney excretes titratable acid, namely, 1, the rate of excretion of buffer; 2, the strength of the buffer acid excreted; and 3, the extent of the depletion of the body stores of available base. The greater the excretion of buffer, the higher the pK' of the buffer acid excreted (within a range of 4.97 to 6.8), and the lower the plasma bicarbonate concentration, the greater is the rate of excretion of titratable acid. The kidney responds immediately and reversibly to changes in these several factors. The manner in which these factors operate to govern the rate of tubular exchange of hydrogen ions for ions of fixed base is considered in the discussion.

REFERENCES

- (1) GAMBLE, J. L. Chemical anatomy, physiology and pathology of extracellular fluid. A lecture syllabus. Department of Pediatrics, Harvard Medical School, Boston, 1941.
- (2) GAMBLE, J. L. *J. Biol. Chem.* **51**: 295, 1922.
- (3) HENDERSON, L. J. *J. Biol. Chem.* **9**: 403, 1911.
- (4) HENDERSON, L. J. *J. Biol. Chem.* **13**: 393, 1913.
- (5) MARSHALL, E. K., JR. *J. Biol. Chem.* **51**: 3, 1922.
- (6) MONTGOMERY, H. AND J. A. PIERCE. *This Journal* **118**: 144, 1937.
- (7) PETERS, J. P. AND D. D. VAN SLYKE. Quantitative clinical chemistry. Vol. 1, Interpretations. Baltimore, Williams & Wilkins Co. 1932.
- (8) PITTS, R. F. AND R. S. ALEXANDER. *This Journal* **142**: 648, 1944.
- (9) PITTS, R. F. AND R. S. ALEXANDER. *This Journal* **144**: 239, 1945.
- (10) PITTS, R. F. AND W. D. LOTSPEICH. *This Journal* (in press).

THE SIMULTANEOUS TRANSPORT OF T-1824 AND RADIOACTIVE RED CELLS THROUGH THE HEART AND LUNGS

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G. N. Stewart (13, 14) devised and developed two methods to estimate the cardiac output from the dilution, by the flowing blood, of a known amount of injected foreign substance. One method, recently revived (15, 9), required determination of the constant concentration in arterial blood of a substance continuously infused into a vein; the other, the mean concentration produced in the first passage of the whole of a single brief and rapid injection.

The basic soundness of the Stewart principles is recognized in their adoption for hydraulic measurements. Their usefulness in the physiological laboratory and the clinic demands the satisfaction of two additional requirements not encountered in pipes and rivers: *a*, there must be no loss of the injected material between the sites of injection and sampling; *b*, the original injection must not be confused with subsequent reinfusions of the material as it is returned to the veins after one or more complete circulations.

Hamilton and his collaborators (7, 8, 10, 11) undertook a series of studies on the validity of the single injection method for determining flow in models, animals and humans. They by-passed the problem of recirculation analytically by extrapolating the early descending points of a semi-logarithmic time-concentration curve. And they also demonstrated that Brilliant Vital Red satisfied the requirement of no loss in transmission, while more diffusible substances were not so reliable. Furthermore, the rapid damping of recirculatory undulations in concentration indicated to them that homogeneous mixing of the injected dye with the circulating blood occurred in a much shorter time than most other workers have believed necessary.

Recent trials (12) of the procedure worked out by Hamilton et al. have brought forth the suggestion that "abnormally high" figures obtained for cardiac output might be due to retention of dye during passage of dyed plasma through the lungs, perhaps because of the theoretical zero velocity and plasma-richness of the peripheral layers of the blood stream along the vascular walls. The development of a technique (3, 4) for identifying injected red blood cells, and for determining them quantitatively through their content of radioactive iron, furnished us with a new angle of attack on these problems: namely, the determination of simultaneous time-concentration curves of tagged cells and dyed plasma after passage of a single rapid intravenous injection through the heart and lungs.

The experiments reported here were undertaken in the hope that they would throw light on two questions. 1. Are the characteristics of the flow through

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the lung vessels such as to cause dye to be retained, either on the vessel walls or in a sluggish layer of plasma along the walls, to give erroneously high estimates of its dilution? 2. Can good evidence be gotten that red blood cells have a slightly more rapid circulation than has plasma as a whole?

METHODS. We originally wished to use the standard procedure (11), with analysis of small samples of arterial blood for both dye and radioactivity, in the hope that the two sets of figures would indicate similar flows. Preliminary calculations, however, showed that the small samples would not furnish enough cells for reliable determinations of radioactivity. It was therefore necessary to do a quick acute experiment, partitioning the total cardiac output into samples for analysis, as long as it would last. This was sure to leave a residuum of both tagged cells and dyed plasma in the undrained thoracic blood. On the other hand, we felt that the alternative of a continued venous supply of blood pooled from other dogs might give hematocrit changes which would introduce even worse complications into the analytical calculations.

To furnish tagged cells for injection, a dog was prepared in the Rochester laboratory as previously described (5). When this donor's blood showed a maximal titer of radioactive iron in the circulating hemoglobin, the dog was shipped to Augusta, where the experiments were done.

The basic procedure was the same in the two experiments. Weighed sampling tubes were mounted on a kymograph with a one-second timer and a signal. A dog was anesthetized with 10 mgm. of morphine sulfate and 170 mgm. of sodium barbital per kgm. The chest was opened in the midline with minimal bleeding, and artificial respiration was instituted. A large copper tube, bent to come up out of the chest and deliver into the sampling tubes, was tied into the brachiocephalic artery at its departure from the aortic arch.

Thirty milliliters of blood was withdrawn from the donor dog (A) and 20 ml. from the operated dog (B). To 25 ml. of the A blood was added 2 mgm. of T-1824 in 1 ml. of 0.9 per cent NaCl, and 20 ml. of this mixture was put in a syringe bearing a large-bore needle. The remainder of the undyed A blood was put in a weighed test tube for later determination of intensity of injected radioactivity.

To 9.0 ml. of B blood was added 1.0 ml. of the dyed A blood. This sample and the remainder of the B blood were centrifuged to get a dyed dilution standard and a dye-free plasma blank.

Three things were now done simultaneously: 1, the kymograph was started; 2, the 20 ml. of dyed A blood was injected as rapidly as possible into the superior vena cava of the operated dog, with a signal on the drum; 3, a large clamp was applied to the aortic arch just beyond the brachiocephalic artery, and the clamp on the latter was released, allowing the whole output of the left heart to be delivered into the sampling tubes in timed succession as they came under the mouth of the copper cannula. When the flow became very slow, the drum was stopped and collection of the drainage was continued in a single tube until it ceased entirely. In the first experiment, a small dog gave 20 samples totaling 138 ml. in about 50 sec.; the second dog gave 40 samples for a total of 342 ml. in 100 sec.

The sampling tubes were wiped and weighed, as much plasma or serum as possible was removed, and the tubes were then tightly stoppered and shipped to Rochester for determinations of radioactivity, while the measurements of dye concentration were done in Augusta.

The red cells were subjected to wet ashing to remove organic matter and the iron precipitated (6). After electroplating the iron (3), activity measurements were made with a modified Geiger-Müller tube employing a scale-of-four circuit. Red cell mass was calculated as described elsewhere (5).

The abundance and the variance of T-1824 methodology in the literature justify a few notes on our analytical procedure. The apparatus employed for photoelectric spectrophotometry incorporates some units and principles whose use is unorthodox but is justified by rigorous tests. The design evolved in a search (in another study) for a combination of stability, high sensitivity, and rapidity of response, and was conditioned by the availability of materials. A Gaertner wave-length spectrometer, fitted with an exit slit, is adjusted to deliver a band about 12 $m\mu$ wide. Between the exit slit and a Photovolt barrier-layer photocell is mounted a closed chamber with a sliding carrier fitted to interpose alternately two matched 6.5 mm. Hellige cells, one for blank solution and the other for unknown. The generated voltage is measured with a L. and N. Student Type potentiometer, using a string galvanometer (string quite loose) as a null point indicator. The logarithm of the ratio of the voltage read with the blank in place to that given by the unknown is taken as the difference in optical density between the two solutions.

Proportionality of voltage and light intensity is not claimed by the manufacturer of the photocell. Nevertheless, readings in microvolts show an excellent linearity up to our maximum output of 10 to 15 mv. when tested with a series of known concentrations of T-1824. For example, we were able (at the time of these expts.) to measure directly optical densities from 0.04 to 2.0 with a maximum deviation of about 1 per cent from the average slope of a calibration line which showed no systematic curvature. We have since narrowed the band to 10 $m\mu$, and this and numerous other improvements have enabled us to better the above precision and extend the range. The instrument has also been used by one of us in studies of absorption spectra of dye-protein combinations reported elsewhere in preliminary form (1). The response of the instrument is so rapid that a 100 watt projection bulb on 60 cycle A.C. power cannot be used for illumination, because the phasic changes in filament temperature are detected by the photocell. Unable to obtain a ribbon filament bulb, we have used a no. 1183 (50 c.p.) spotlight bulb operated at 6 or 8 volts, depending on the part of the spectrum needed.

Prevention of coagulation presented difficulties only because the unpredictably variable size of the samples precluded a quantitatively similar treatment of standards and all samples. In the first experiment powdered heparin was added to the blood initially drawn from both dogs; the later samples were allowed to clot, furnishing serum for the dye determinations. The necessary spectrophotometric correction for the heparin (an inferior preparation—better ones are now obtainable) was appreciable, though small, and was properly applied after

determination of the absorption spectrum. In the second experiment the initial withdrawals of blood from both dogs were made into 1.0 ml. of 1.7 per cent potassium oxalate solution, and 0.2 ml. of the same oxalate was added to each of the sampling tubes on the kymograph. The volumes of blood received by these tubes varied, with the rate of flow, from 5 ml. to 22 ml. Coagulation was adequately inhibited in all but the largest samples, and corrections were applied for the calculated dilution of both plasma color and dye. It was necessary to assume that the amount and concentration of oxalate used would not appreciably affect the relative cell volume.

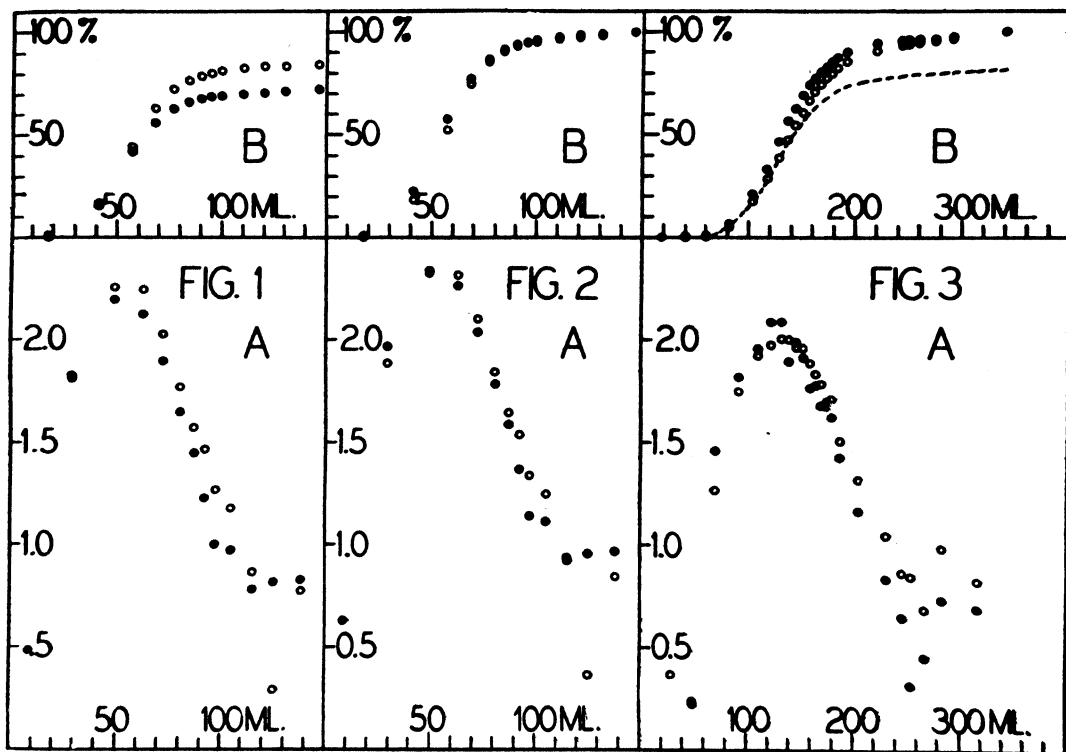
Some hemolysis was present in practically all tubes of both series and, however slight, was not neglected. Optical densities were determined at 540 and 620 $m\mu$ on all samples, on dye alone in plasma, and on dye-free slightly hemolyzed plasma which had stood just as long as the samples. We have not found the importance of this time factor emphasized in previous work on corrections for hemolysis, though the spontaneous interconversions of hemoglobin derivatives responsible for it have been investigated (2) and are well known to students of the blood pigments. Quite possibly, the filters of the commonly used photoelectric colorimeter do not distinguish between the hemoglobin forms enough to produce a large change of the correction factors with time; but if a narrow spectral band is used, the density ratios will depend on the band width and on the age and concentration of the hemoglobin solutions. All of our determinations were carried out after the samples had stood in the refrigerator over night, at which time the rate of further change should be negligible. The optical density ratio $\frac{540}{620}$ for the hemoglobin at this time was 10.7, whereas a fresh sample

showed a rapidly falling ratio (caught once at about 32). The corresponding ratio for the dye was determined to be 0.4. The hemolysis correction was made by the commonly used method, employing the simultaneous equations which result from the measurements at 540 and 620 $m\mu$ and from the assumption that the optical density of a mixture at any given wave length is the sum of the densities of its components at that same wave length.

Volumes of the samples were taken as their weights divided by 1.06. It should be noted that the method for determining tagged cells gave the total amount of radioactivity in each sample and in an aliquot of the injected blood, with relative concentration being calculated from these figures and the volume; while the dye method gave concentration directly in milligrams per milliliter of blood, from which figure and the volume the total amount of dye was calculated. These methods require no determination of the hematocrit, but only an assumption of its constancy.

RESULTS. Neither of the two experiments was perfect in itself: in the first we were disappointed in the short duration and the small total volume of flow and number of samples obtainable; in the second these were much improved but an error in the dilution standard (irretrievable when detected) made the absolute figures for dye concentration impossibly high. Presumably, however, relative concentrations were reliable. Taken together, we regard the two experiments as significant.

The results are presented in figures 1 to 3. In each, the abscissa is divided in units of accumulated volume rather than time, in order to compensate for the varying rate of flow. In the A portion of each figure, the ordinates are logarithmic representations of concentrations of T-1824 and tagged cells per milliliter of whole blood, adjusted to numerical comparability on the basis of the amount injected or recovered. The B portion of each figure shows the accumulated recovery of dye and tagged cells, sample by sample. The points in the B parts of



Figs. 1-3. Explanation in text

the figures are placed corresponding to the accumulated flow at the end of each sample; in the A parts, corresponding to the middle of each sample. Throughout, solid dots represent radioactive cells, circles T-1824.

In figure 1, the first experiment is plotted with proportional concentrations and recoveries calculated on the basis of the amount of dye and radioactivity injected. Figure 3 shows the results of the second experiment, with the dotted line of the B portion calculated as in figure 1. Because of the failure of the absolute figures for dye, the rest of figure 3 is plotted on the basis of recovered dye and cells rather than injected amounts. Figure 2 gives the results of the first experiment plotted the same way, for comparison with figure 3.

In regard to the deviations of the late points and some of the earliest ones, it must be remembered that at the bottom of a logarithmic plot they represent

actually quite minor aberrations. The lowest of these points correspond to concentrations that are on the borderline of analytical reliability.

DISCUSSION. Comparing the aim of these experiments with the results presented in the figures, we regard three points as significant:

1. As shown in figure 1-B (and supported logically by the similarity between figs. 2 and 3), there is certainly no evidence that (at the dye concentration used) there is any preferential retention of dyed plasma in the vessels of the lesser circulation.

2. The slightly earlier peak and decline of the concentration curves for radioactivity indicate that through at least some types of vessels the velocity of the cells is significantly greater than that of the plasma.

3. When allowance is made for this slight and fairly constant lead, the simultaneous concentration curves for cells and dye are practically identical: thus the transport and delivery of tagged cells and of labeled plasma show the same dependence upon blood flow.

The injection of dye for a determination of blood volume is commonly much slower than can be used in blood flow measurements, where it is necessary to get a clearly defined peak and decline of concentration before the onset of recirculation. One of us (5) has pointed out that in the blood volume procedure the sluggish layers of plasma, far from trapping dye, should be expected to get less than their due share at first. *A priori*, a similar argument in the case of the rapid injection is not so cogent. But the present experiments indicate that such retention of dye cannot be quantitatively important in this method either, at the concentrations employed.

It is obvious that these experiments, which leave some 15 per cent of the injected dye and roughly 20 per cent of the injected cells unaccounted for, cannot serve as convincing examples of the efficacy of the injection method. This was a necessary sacrifice to avoid various sources of technical difficulty, since the particular combination of requirements introduced an experimental strain at every step. It seems a most natural assumption that both dye and cells should be trapped in circuits through which flow stops completely as the total flow dwindles. From this viewpoint the linearity of the logarithmic decline of concentration, and its resemblance to the picture obtained with a constant flow, are only a coincidence. It is the essential identity of the simultaneous concentration curves for dye and cells that leaves us convinced that as far as retention in the pulmonary system is concerned the intravascular dyes are adequate for the estimation of blood flow.

SUMMARY

1. After rapid injection (superior vena cava) of red cells tagged with radioactive iron in plasma dyed with T-1824, the entire left ventricular output was collected in a series of measured and mechanically timed samples, which were analyzed for dye and for radioactivity.

2. Recovery of neither cells nor dye was complete, probably because of total collapse of some vascular circuits with diminishing flow.

3. However, there was no evidence that in comparison with the cells there was any preferential retention of dye (at the concentration used) in or on the vessels of the lesser circulation.

4. The simultaneous concentration curves for dye and tagged cells were practically identical, with the curve for cells always a few milliliters of flow ahead, indicating a more rapid transit of the cells in some part of the circuit.

5. It is concluded that the "high" cardiac outputs obtained by the injection method are not in error as the result of retention of dye in the lungs.

REFERENCES

- (1) DOW, P. Fed. Proc. **4**: 16, 1945.
- (2) DRABKIN, D. L. AND J. H. AUSTIN. J. Biol. Chem. **98**: 719, 1932.
- (3) HAHN, P. F. Ind. and Eng. Chem., Anal. Ed. **17**: 45, 1945.
- (4) HAHN, P. F., W. M. BALFOUR, J. F. ROSS, W. F. BALE AND G. H. WHIPPLE. Science **93**: 87, 1941.
- (5) HAHN, P. F., J. F. ROSS, W. M. BALFOUR, W. F. BALE AND G. H. WHIPPLE. J. Exper. Med. **75**: 221, 1942.
- (6) HAHN, P. F., W. F. BALE, E. O. LAWRENCE AND G. H. WHIPPLE. J. Exper. Med. **69**: 739, 1939.
- (7) HAMILTON, W. F., J. W. MOORE, J. M. KINSMAN AND R. G. SPURLING. This Journal **84**: 338, 1928.
- (8) HAMILTON, W. F., J. W. MOORE, J. M. KINSMAN AND R. G. SPURLING. This Journal **99**: 534, 1932.
- (9) HOLT, J. P. This Journal **142**: 594, 1944.
- (10) KINSMAN, J. M., J. W. MOORE AND W. F. HAMILTON. This Journal. **89**: 322, 1929.
- (11) MOORE, J. W., J. M. KINSMAN, W. F. HAMILTON AND R. G. SPURLING. This Journal **89**: 331, 1929.
- (12) SMITH, H. W., S. E. BRADLEY AND E. E. SELKURT. Personal communication.
- (13) STEWART, G. N. J. Physiol. **22**: 159, 1897.
- (14) STEWART, G. N. This Journal **57**: 27, 1921.
- (15) WIGGERS, H. C. This Journal **140**: 519, 1944.

FURTHER OBSERVATIONS ON THE DISTRIBUTION AT THE LEVEL OF THE PONS OF DESCENDING NERVE FIBERS SUBSERVING HEAT REGULATING FUNCTIONS¹

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The interpretation of results of experiments designed to map the distribution of nerve fibers subserving heat regulating functions as they descend through the pons and upper medulla has been clouded by indications that a subsidiary heat maintenance center might be located at this general level (1, 2, 3).

The existence of a subsidiary pontine or upper medullary heat maintenance center has long been postulated because hyperthermia has frequently been associated with transections (acute experiments) and tumors at this general level. So far as we have determined, Tscheschichin (4) first described hyperthermia following a transection of the brain stem through the caudal pons.

Tscheschichin did not give the environmental temperature to which his animals were subjected, but it is assumed the experiments were performed in ordinary laboratory temperatures. He reported, however, that hypothermia usually followed what seemed to be homologous transections. This also has been our experience both in the research and teaching laboratories.

Why does hyperthermia at times and hypothermia at other times follow transections which appear to be anatomically homologous? Is the hyperthermia due, perchance, to the release of a subsidiary thermogenic center in the upper medulla or lower pons, or is it due to so-called irritative stimulation of cells located at this level but possessing mere synaptic functions in the descending thermogenic pathways?

This same problem is strikingly presented when heat maintenance powers return, after temporary elimination, following an incomplete pontine or upper medullary transection. In this situation the question arises as to whether the return of these powers is due to activity of a released subsidiary center or to recovery of function of traumatized fibers subserving heat maintenance functions which descend through the unsevered tissue. This particular problem was resolved by placing an opposite hemisection of the brain stem at a slightly higher level, so as to complete the transection, thereby reducing the animal to a modified pontine preparation. The results obtained are described and discussed in the succeeding pages.

METHODS. The selection and nursing care of animals, surgical approaches to the brain stem, and the handling and preparation of tissues have been described in detail in a recent communication (5).

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The incomplete transection was accomplished surgically in the following way: Following a unilateral exposure of the corpora quadrigemina, a probe was projected ventrocaudally, pressed to the desired distance across the midline, and then pulled back (laterally) through the soft tissue of the pons. In this way, in addition to executing a complete hemisection of the brain stem on the side of the exposure, the medial portion of the opposite half-section of the pons was also severed. The amount of infringement upon the opposite half-section varied according to the pressure applied to and the slant given the probe toward the

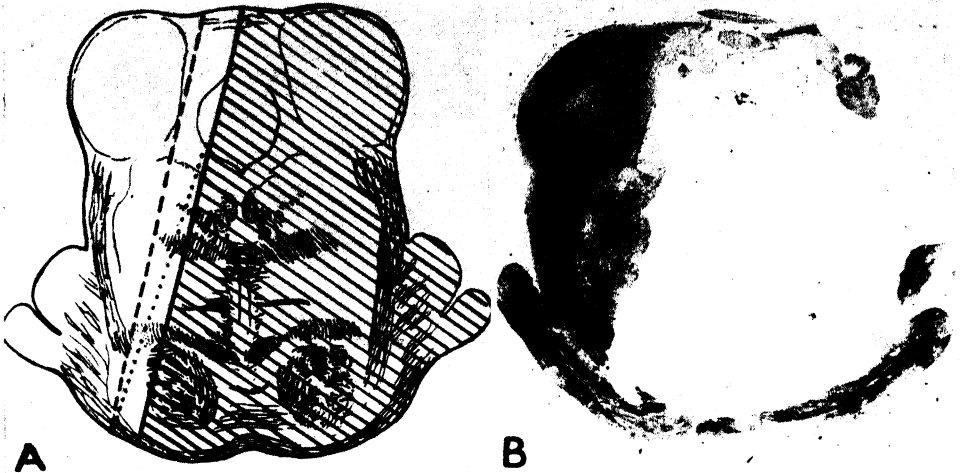


Fig. 1. A. Drawing of a cross section of a dog's brain stem, cut, on a slant dorsoventrally, through the middle of the inferior colliculi dorsally and the middle of the pons ventrally. The lined area to the right of the heavy semivertical line approximates the location and extent of the tissue of the pons which was severed; that to the left the area which remained unsevered in dog 1. The area to the right of the dotted line approximates the portion of the tissue which was severed; that to the left the area which remained intact in dog 2. The area to the right of the broken line approximates the portion of the pons tissue which was severed; that to the left the tissue which remained anatomically intact in dogs 3 and 4.

B. Photomicrograph of a cross section taken from the series on dog 3 through the region where the lesion attained its greatest width showing the actual amount of tissue which remained unsevered. Note that the extreme lateral bit of the medial lemniscus remained unsevered and that only that portion of the lateral reticular formation remained which lies completely lateral to the brachium conjunctivum, which structure was completely severed.

opposite side. Different degrees of incomplete transections accomplished in this way are illustrated in figure 1.

Following this operation, the animal's heat regulating abilities were assayed periodically until normal performance was attained or until a steady state was reached, as determined by the assay methods previously described (6).

If recovery of function occurred, a second operation was performed in which the transection was completed by a second hemisection placed on the opposite side at a slightly higher level. Accomplishment of such a two-stage operation is illustrated in the photographs of sections taken from the series on dog 1 shown in figure 2.



Fig. 2. Photomicrographs of representative frontal sections taken from the series on dog 1, showing the actual location and extent of the two transverse lesions which obtained in this animal. The lesion on the right was inflicted at the first operation, the one on the left 8 weeks after the first.

A is a dorsal section which cuts through the lateral walls of the fourth ventricle. Prominent localizing structures are the vestibular nuclei, the brachia conjunctiva, and the brachia of the inferior colliculi.

B is a more ventrally located section, though it also cuts through the dorsal aspect of the brain stem. Note particularly the facial genua, the red nucleus on the right side, and the dorsal aspect of the hypothalamic gray.

C is a section through approximately the middle of the brain stem. Note the relation of the right lesion to the brachia of the inferior colliculi, the brachia of the pons, and the facial nerves; also the relation of the left lesion to the oculomotor filaments on the right side and the hypothalamic gray as a whole.

D is a ventrally situated section in which the superior olives and the substantia nigra

RESULTS. Hemisection of brain stem. Heat regulating powers were never disturbed, even temporarily, when the transverse lesion was restricted to a hemisection of the brain stem at any level of the midbrain, pons, or upper medulla. No asymmetry could be detected between the two sides of the body in the caliber of the blood vessels, or in shivering. We have repeatedly verified our original observations to this effect (7).

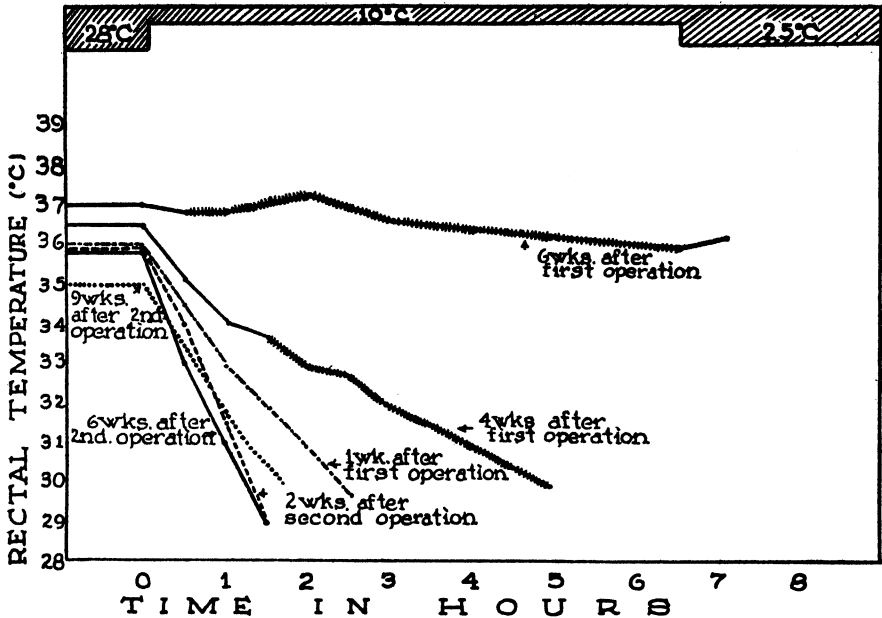


Fig. 3. This is a composite chart showing the rectal temperature curves of dog 1 during the 10°C. environmental temperature tests made following the right and left transverse lesions shown in figure 2. The crossed lines superimposed on the rectal temperature curves indicate the time when it was actually observed that the animal was shivering. The pre-test environmental temperature of 25°C. obtained for the fourth and sixth weeks' tests run the fourth and sixth post-operative weeks, rather than the 28°C. indicated. Also, the pre-test environmental temperature for the ninth week test after the second operation was 27°C.

The solid line to the extreme left of the figure is the curve for the test carried out 6 weeks after the second operation; the broken line is the curve for the test run 2 weeks after the second operation; and the dotted line the curve for the test carried out 9 weeks after the second operation. The labeling for the tests carried out after the first operation is self-evident.

Return of heat maintenance functions after temporary elimination by an incomplete transection. The heat maintenance ability was not impaired until a considerable amount of the medial portion of the opposite half-section of the pons was involved, i.e., in addition to a complete hemisection. Of considerable importance is the fact that even when lesions are sufficiently extensive to precipitate partial deficits, there has been no detectable evidence of asymmetrical control

on the two sides of the body. Maximal temporary impairment of heat maintenance ability with subsequent recovery to normal was encountered in dog 1. This dog's postoperative responses to 10°C. environmental temperature tests carried out the first, fourth, and sixth weeks after the first operation are illustrated in figure 3. These curves demonstrate a relatively slow but progressive return over a period of six weeks from no heat maintenance ability to essentially normal function. The response to the 10°C. test during the eighth postoperative week, not illustrated, was within the normal range of responses. The extent of the incomplete transection at four frontal levels is shown in the photographs in figure 2, and its entire extent is indicated by the lined area on the cross-section schema, shown in figure 1.

One week after operation this dog's rectal temperature was maintained at 36°C. when housed in a 28°C. incubator. Its regulation interval was thus 8°C. In the 10°C. test the rectal temperature fell from 36°C. to 29.7°C. in 2½ hours, with no shivering.

Four weeks after operation, the regulation interval had increased to 8.5°C. At this time the animal combatted the 10°C. test for 5 hours before the rectal temperature was reduced to 30°C. Shivering appeared when the rectal temperature reached 34°C. and continued throughout the test. It is strikingly noticeable that the presence of shivering lessened the rate of fall in rectal temperature.

Six weeks after operation, the dog maintained a rectal temperature of 37°C., only one degree below normal, when housed in ordinary room temperature (25°–26°C.). It also successfully combatted the 10°C. temperature for a period of 6½ hours with a lowering of rectal temperature only from 37°C. to 36°C. Shivering appeared almost immediately and continued throughout the test.

Eight weeks after operation, when the dog was subjected to the 10°C. test for an 8-hour period, the rectal temperature fell only 1°C., from 38°C. to 37°C. Bilateral shivering was present throughout the test.

The heat loss mechanism was not disturbed in this animal as evidenced by the fact that it was able to combat a 38°C. environmental temperature for 3½ hours in an entirely normal manner. The rectal temperature curve during the 38°C. test carried out the second week after operation is shown in figure 4.

Complete and permanent elimination of heat maintenance and polypneic functions following completion of the incomplete transection by an opposite hemisection. The transection of the brain stem was completed in dog 1 by an opposite (second) hemisection eight weeks after the first transverse lesion was placed (see fig. 2). It will be noted from figure 3 that this procedure completely and permanently eliminated the animal's ability to combat a cool environment as evidenced by 10°C. tests performed the second, sixth, and ninth weeks after this second operation. Note the very slight but detectable decreased rate of fall in rectal temperature during the ninth week test as compared with the second and sixth weeks' tests. This is believed to be evidence of a low-grade vasomotor adjustment present in chronic low pontine preparations but absent in chronic midbrain or hypothalamic preparations.

The second hemisection also eliminated the polypneic response to overheating, which remained normal following the first transverse section. This is shown in figure 4 by the rectal temperature curve during the 38°C. test run the third week after the second operation. Note that polypnea and panting did not occur, although the rectal temperature rose to 43°C. There was, however, a detectable non-polypneic reaction against over-heating as shown by a much slower rise in rectal temperature than occurs in certain acute low midbrain preparations where the rise in rectal temperature progresses rapidly along a straight line. This was obviously the result of adaptation in the vasomotor outflows. For comparative

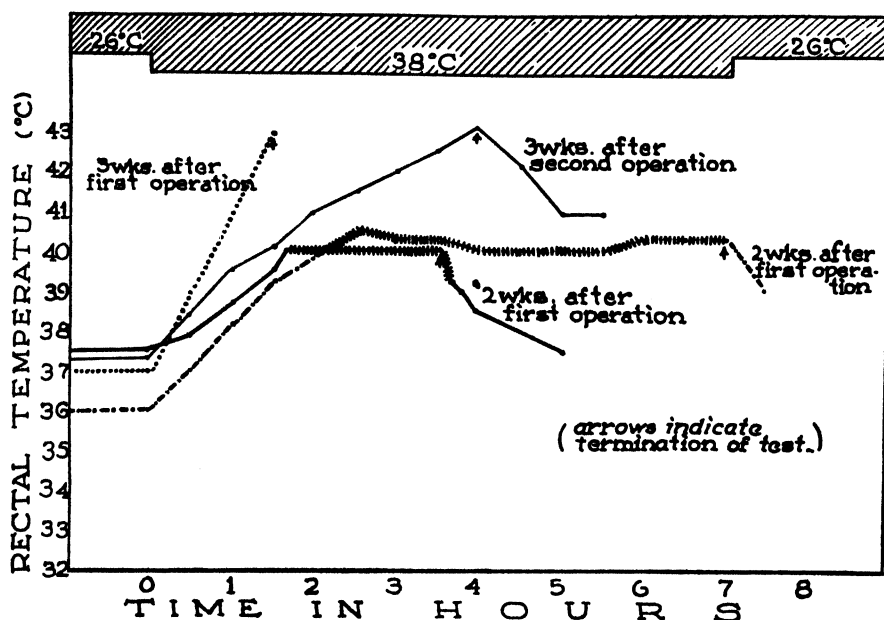


Fig. 4. This is a composite chart showing the rectal temperature curves for dogs 1, 3, and 5 during 38°C. environmental temperature tests. The solid lines are the curves for dog 1, the broken line for dog 3 and the dotted line for dog 5. The crossed lines superimposed on the rectal temperature curves indicate the time when panting was actually observed to be present. The pre-test environmental temperature of 26°C. as indicated in the chart is incorrect in all instances; the temperature which obtained ranged from 28°-30°C.

purposes, such a test is illustrated by the rectal temperature curve for dog 5, an acute low midbrain animal, in figure 4 (dotted line).

Permanent elimination of heat maintenance, with sparing of heat loss functions, by an incomplete transection. The extent to which the medial portion of the opposite half-section of the brain stem must be involved to eliminate permanently heat maintenance ability was shown in dog 2. The extent of the lesion is indicated by the dotted line in the schema in figure 1A. This dog exhibited a barely detectable possible remnantal heat maintenance control in that in the 10°C. tests which were run during the second, fifth, and seventh postoperative weeks, it

required slightly longer to lower the rectal temperature to 30°C. than was the case in the final tests on dog 1. However, during all the tests, the rate of fall was along a straight line, and there was no shivering. It required 2½ hours in the second and fifth weeks' tests, and 3 hours during the seventh week test to lower the rectal temperature from 37°C. to 30°C.

This dog's heat loss functions were not affected by the lesion, as evidenced by the fact that the animal regulated normally in 38°C. environmental temperature tests run during the second and sixth postoperative weeks. During the sixth week test, panting began when the rectal temperature reached 39°C. and polypnea began at a rectal temperature of 39.7°C. The maximal rise in rectal temperature during the test, which was continued for 6 hours, was to 40.2°C. At the end of the six-hour period, the rectal temperature had fallen to 39.6°C.

The extent to which the medial part of the opposite half-section of the pons can be involved, in addition to a hemisection, without impairing heat loss functions, is shown in dog 3. The photograph of a section from the series on this dog is reproduced in figure 1B, and the extent of the lesion is indicated by the broken line on the schema in figure 1A. This dog's response to a 38°C. environmental temperature test run two weeks after the operation is illustrated in figure 4. It will be noted that polypnea began at a rectal temperature of 40°C., and the maximal rectal temperature reached was 40.3°C. during a 7-hour test. This dog did not survive long enough for the heat maintenance assay studies.

It is possible, however, for the adequacy of over-all heat loss functions to be impaired both temporarily and permanently by an incomplete transection which appears to be anatomically homologous with the lesion which obtained in dog 3. For example, complete elimination of heat maintenance ability with a definite impairment of heat loss functions obtained in dog 4. The extent of the incomplete transection was essentially identical with that of dog 3 indicated in the schema in figure 1A. Accordingly it is assumed that in this instance (dog 4), some of the heat loss fibers descending in the unsevered tissue were permanently paralyzed by the trauma sequelae incident to the lesion. This dog was subjected to a 37°C. environmental temperature test on the second and fifth postoperative weeks. In both instances, polypnea began and appeared typical when the temperature reached the neighborhood of 40°C. Nevertheless, the rectal temperature continued to rise progressively, with only a slight transitory tendency to level off. The rectal temperature reached 42.5°C. in 4 hours in the second week test and 43°C. in 5 hours during the fifth week test, at which time each test was terminated. Thus over-all heat loss function was inadequate, although all individual mechanism outflows outwardly appeared normal.

DISCUSSION. *Subsidiary center versus temporary traumatic paralysis of descending fibers.* The problem of determining whether the return of heat maintenance functions following an incomplete transection was due to progressive activation of a released subsidiary center or to recovery of function in traumatized fibers passing through the unsevered tissue was resolved by a two-stage operation whereby opposite hemisections were placed at slightly different levels. The results are well demonstrated and illustrated by the experiment on dog 1.

The first (right) lesion (see fig. 2) in this dog eliminated heat maintenance powers only temporarily, with a progressive postoperative return to normal. Completion of the transection by the opposite (left) hemisection permanently eliminated heat maintenance powers (see fig. 3). We interpret these results as conclusive evidence that the return of heat maintenance powers following the first transverse section was actually due to recovery of function of temporarily paralyzed nerve fibers descending laterally through the unsevered tissue.

It should be pointed out, however, that this type of experiment, i.e., one having opposite transverse sections, does not rule out the possibility of the existence of a subsidiary center at a more caudal level than the right transverse section illustrated in figure 2.

Demonstrated functional neighborhood effects of an acute lesion in the central nervous system. The temporary elimination of heat maintenance powers by the first transverse section in dog 1 demonstrates conclusively the grave functional effects that an acute lesion in the central nervous system may have on the neurological elements in the immediate environs of the lesion. In this connection an important working criterion to be derived from this investigation is that in localization studies on heat regulation a preparation must be maintained at least six weeks, preferably eight weeks, after operation before the residual deficits may be evaluated with certainty. This is readily evident from the results described and illustrated for dog 1. We originally thought it safe to evaluate residual deficits on preparations maintained only four weeks (6). It may not be safe to assume that the entire recovery process was due to clearing of the lesion trauma sequelae. It would seem possible that a markedly reduced number of neurons might make some compensatory adjustment during the later period of the over-all recovery process. For instance, it is conceivable that the improvement occurring between the fourth and sixth weeks might have been wholly due to true compensatory processes. Yet there is some doubt that such compensation occurs since incomplete deficits occur and persist permanently following certain types of lesions in the hypothalamus (6). The slow progressive type of recovery which occurred in the instance of dog 1 is entirely similar to the rate and character of the return of function which occurs in certain pressure or traumatic syndromes encountered in peripheral nerve injuries where compensation as such seems not to complicate the picture.

Neurological separation of descending fibers subserving heat maintenance and heat loss functions. A former observation, namely, that heat maintenance powers can be permanently eliminated with a sparing of the heat loss mechanism by appropriate pontine medial transverse lesions was fully substantiated in this study. This is effectively demonstrated by the result described for dog 2. The extent to which an incomplete transection may reach without affecting, even during the acute stage, the adequacy of the heat loss mechanism, was shown in the instance of our dog 3 (see fig. 1A). Certainly some of the heat loss fibers have an *extreme lateral* distribution at the level of the pons.

The fact that intact heat loss powers following an extensive transverse section are also dependent on fibers actually descending through the unsevered tissue is

evident since heat loss powers—like heat maintenance powers—were permanently eliminated when the transection of the brain stem in dog 1 was completed by the opposite hemisection.

SUMMARY

Information regarding the lateral distribution of descending nerve fibers subserving heat regulating functions was gained by studying dogs for extended periods after placing graded transverse lesions in the pons in the manner illustrated in figure 1.

In addition to a complete hemisection on one side, a transverse section must extend across the midline and involve a considerable portion of the medial tissue of the opposite half of the stem before the animal's heat regulating ability is in any way affected. When a deficit obtains, there is no grossly detectable asymmetry in the neurogenic outflows between the two sides of the body.

When sufficient of the opposite medial tissue is involved to affect heat regulation, the heat maintenance mechanism is impaired or permanently eliminated (depending on the width of the lesion) before heat loss ability is detectably altered. Accordingly, although both types of fibers are distributed well laterally, in a cross section of the pons, the heat loss fibers have a more lateral distribution than do the heat maintenance fibers.

The retention of either heat maintenance or heat loss powers in chronic preparations following an incomplete transection of the pons is dependent on the functional activity of nerve fibers descending through the unsevered tissue, and not to the activity of medullary subsidiary neurons. This is evident from the fact that these functions are eliminated permanently when the transection is completed by an opposite hemisection of the midbrain as shown by the photographs in figure 2 and the assay of heat regulating functions illustrated in figures 3 and 4.

Of considerable significance to localization studies is the fact, illustrated particularly well in the experiment on dog 1, that descending fibers which subserve heat regulating functions may be temporarily paralyzed by the trauma sequelae of an acute lesion. *The assay of heat regulation deficits on acute or subchronic preparations is therefore entirely unreliable.*

REFERENCES

- (1) KELLER, A. D., AND W. K. HARE. Proc. Soc. Exper. Biol. and Med. **29**: 1067, 1932.
- (2) KELLER, A. D. Am. J. Med. Sci. **135**: 746, 1933.
- (3) KELLER, A. D. J. Neurophysiol. **1**: 543, 1938 (see footnote on p. 555).
- (4) TSCHESCHICHIN, J. Arch. f. Anat., Physiol. u. Wissench. Med., Leipzig, 151-179, 1866.
- (5) KELLER, A. D. J. Neurophysiol. **8**: 275, 1945.
- (6) BLAIR, J. R. AND A. D. KELLER. J. Neuropath. and Exper. Neurol. **5**: 240-256, 1946.
- (7) KELLER, A. D. This Journal **93**: 665, 1930.

THE EFFECT OF VARIOUS IONS ON THE ANAEROBIC GLYCOLYSIS OF RAT LIVER *IN VITRO*^{1, 2}

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The anaerobic carbohydrate metabolism of rat liver *in vitro* presents certain peculiar features. First of all, the liver is characterized by its ability to form two end-products, glucose and lactic acid, from the anaerobic breakdown of glycogen, and there is little information from *in vitro* studies as to the usual proportions of these substances formed. Also, as regards lactic acid formation (anaerobic glycolysis), there is disagreement in the literature as to the magnitude of this function when measured *in vitro* in the usual Ringer-bicarbonate medium. Burk (1) states that in his experience the $Q_{O_2}^{N_2}$ is seldom above 1.0, while Orr and Stickland (2, 3) report values as high as 10–15 depending upon the glycogen content of the slices. In further contrast to most tissues, the anaerobic glycolysis of rat liver decreases rapidly with time (4, 6) and is essentially uninfluenced by the presence or absence of glucose in the medium (2, 4, 5). Recently, certain features of rat liver metabolism *in vitro* have been shown to be influenced favorably by suspending the tissue in media in which the cation balance resembles that of intracellular fluid. Hastings and Buchanan (7) used one such "intracellular" medium to demonstrate glycogen synthesis from glucose, a process which rat liver fails to exhibit in the usual Ringer-bicarbonate medium, while Fuhrman and Crismon (8) employed another "intracellular" medium to secure an increased ability of the liver to recover its oxygen consumption after exposure to anaerobic conditions. We have consequently thought it worthwhile to investigate the effect of these media, and certain modifications of them, on rat liver anaerobic glycolysis and sugar formation, and the results form the basis of this report.

METHODS. The white rats used were of either sex, weighed from 200 to 280 grams and were fed Purina feed pellets *ad libitum*. They were killed by a blow on the head and cutting the throat, and the liver was sliced by razor into a Petri dish containing iced Ringer solution. (Substituting intracellular medium for the Ringer solution in the dish was found to be without effect.) The slices were blotted on filter paper and weighed on a torsion balance; "initial dry weight" was calculated from the wet weight by dividing by the factor 5. The samples were then incubated in Warburg vessels at 38°, having been equilibrated with the proper gas (nitrogen or 95 per cent nitrogen—5 per cent CO₂ passed over hot copper to remove traces of oxygen). One set of vessels was removed at the end of the equilibration period for the "initial" analyses and the other set one hour later for the "final" analyses. Manometric readings were taken during this

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² A preliminary report of these results has appeared in Fed. Proc. 5: 110, 1946.

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interval, and, when corrected for the retention of CO_2 in the medium, agreed well with the chemical estimations of lactic acid production. Nearly all the data reported in this paper, however, are based on the chemical analyses, in some cases expressed in manometric terms for comparative purposes. The method of Barker and Summerson (9) was used for lactic acid analysis, that of Good, Kramer and Somogyi (10) for glycogen, and the Benedict (11) or Folin-Malmros (12) methods for reducing sugar, as will be indicated. The lactate and sugar analyses were performed on aliquots of the suspension medium, whereas the glycogen analyses were made on the remaining medium and tissue, control experiments having demonstrated that only an insignificant amount of glycogen

TABLE 1

Ionic composition of the various media in millimoles per litre

MEDIUM	Na	K	Ca	Mg	Cl	PO_4	HCO_3	SO_4
Ringer-bicarbonate.....	146	2.5	1.7	0	127	0	25	0
Fuhrman and Crismon Media								
Extracellular*.....	157	2.4	1.7	0.8	127	20	0	0
Intracellular.....	0	121	0	18.5	0	75.5	0	10
Intracellular with added bicarbonate†.....	0	146	0	18.5	0	75.5	25	10
Hastings and Buchanan media								
Extracellular.....	152	5	1	1	121	0	40	0
Intracellular.....	0	130	0	20	130	0	40	0
Intracellular with added phosphate††.....	0	107	0	20	40	67	40	0

* This medium is quite properly not referred to by Fuhrman and Crismon as representing the composition of extracellular fluid. It is a Ringer-phosphate medium previously employed by Craig (13).

† Our modifications.

‡ This composition results from replacing KCl of the Hastings and Buchanan medium with the same amount of potassium phosphate buffer mixture as is used in the Fuhrman and Crismon intracellular medium.

appears in the medium under these conditions. All analyses were done in duplicate, either on duplicate or triplicate samples of tissue.

The ionic composition of the various media used in this study is given in table 1. It will be noted that the Fuhrman and Crismon media are heavily buffered with phosphate and lack bicarbonate, whereas the reverse is true of the Hastings and Buchanan media. In each case, the chief features of the intracellular media are the substitution of potassium for sodium ions, the absence of calcium, and the addition of more magnesium ions. Our modifications of these media are such as to result in solutions rich in potassium, phosphate and bicarbonate ions. It will be pointed out further on that these three ions exert important effects on rat liver anaerobic glycolysis, whereas the other components of the media appear to be of subsidiary importance in this respect. Glucose was added to the medium only as indicated below.

RESULTS. In table 2 are listed the anaerobic glycolysis figures obtained with the tissue suspended in the phosphate media of Fuhrman and Crismon and also in their intracellular medium to which 25 mM/litre of sodium bicarbonate⁴ was added. The somewhat higher values of lactic acid production in the intracellular medium are clearly augmented still further by the addition of bicarbonate

TABLE 2
Anaerobic glycolysis in the phosphate media of Fuhrman and Crismon

EXPT. NO.	$Q_G^{N_2}$ IN THE FOLLOWING MEDIA		
	EXTRACELLULAR	INTRACELLULAR	INTRACELLULAR PLUS BICARBONATE
23	0.5	4.6	10.9
24	2.3	5.1	8.5
25	0	3.5	10.6
43	2.0	2.8	5.8
44	1.1	2.5	5.0
Average.....	1.2	3.7	8.2

TABLE 3
Relationship of glycogen breakdown to lactic acid formation in the Fuhrman and Crismon media
(The figures represent averages for the five experiments listed in the previous table)

	EXTRACELLULAR MEDIUM	INTRACELLULAR MEDIUM	INTRACELLULAR PLUS BICARBONATE MEDIUM
A. Initial glycogen (per cent of wet weight).....	3.0	2.7	2.7
Glycogenolysis in 1 hour			
B. in gamma/mgm. tissue.....	37	53	80
C. as per cent of initial glycogen.....	24	39	59
Lactic acid formation			
D. in gamma/mgm. tissue.....	5	15	33
E. as per cent of glycogenolysis.....	13	28	41
Unaccounted-for fraction of glycogenolysis			
F. in gamma/mgm. tissue.....	32	38	47
G. as per cent of glycogenolysis.....	87	72	59

equilibrated with 5 per cent CO₂. In interpreting these results, it was desirable to determine how the effects of the media are related to the glycogen content of the slices and to glycogenolysis. This information is given in table 3, which presents further data obtained in the same experiments shown in table 2. The glycogen analyses of the liver slices at the beginning of the experimental period are

⁴ Potassium bicarbonate was used in control experiments and produced the same effects.

shown in row A; they are so nearly the same that the effect of the media is clearly not attributable to differences in the initial glycogen content of the slices. The differences between the initial and final glycogen analyses are expressed in row B as the micrograms of glycogen broken down per milligram dry weight of tissue and in row C as the per cent of the initial glycogen disappearing during the experimental period. The relative rate of glycogenolysis in the three media is similar to that shown above for anaerobic glycolysis, being greatest, both in absolute and relative terms, in the intracellular-plus-bicarbonate medium. Row D indicates the glycolysis in micrograms of lactic acid formed per milligram dry weight of tissue, and in row E this is expressed as the percentage of the glycogen breakdown accounted for by this lactate formation. Finally, the unaccounted-for fraction of the glycogen broken down is shown in micrograms/milligram tissue (row F) and as the per cent of glycogenolysis (row G). It will be noted that the absolute amount of this fraction is greatest but the relative amount least in the intracellular-plus bicarbonate medium, with intermediate values in the intracellular medium. The nature of this fraction was investigated in the ex-

TABLE 4

Formation of lactic acid plus reducing sugar as per cent of glycogen breakdown

MEDIUM	NO. OF EXPTS.	LACTIC ACID PLUS REDUCING SUGAR AS PER CENT OF GLYCO- GEN BREAKDOWN
		%
Ringer-bicarbonate.....	2	84
Fuhrman and Crismon extracellular.....	1	89
Fuhrman and Crismon intracellular with added bicarbonate.....	1	99
Hastings and Buchanan intracellular with added phosphate.....	2	96

periments shown in table 4. It was of course inferred that the material is glucose, but there was also the possibility that phosphorylated hexoses are included. Accordingly, in these experiments, in addition to the glycogen and lactic acid analyses, reducing sugar was determined by the method of Folin and Malmros, a method with which the phosphorylated hexoses exhibit considerably less than the full reducing power of glucose (14). If there had been an appreciable accumulation of these intermediates, a marked discrepancy would be evident between the glycogen broken down and the sum of lactic acid plus sugar formed. We interpret such discrepancies as were found (cf. table 4) to be within the limits of experimental error, although this is perhaps open to question in the experiments in Ringer-bicarbonate medium. In general, however, the data suggest little if any accumulation of phosphorylated intermediates.

Our findings to this point consequently show that as glycogenolysis is increased by changes in the ionic composition of the medium, the formation of both reducing sugar and lactic acid is increased, but particularly the latter, so that the dual pathway of glycogen breakdown is shifted in the direction of enhanced lactic acid

formation. It has also been shown above that the effects of the various media cannot be attributed to differences in the glycogen content of the liver slices at the beginning of the experimental period. It should now be made clearer, however, that the glycogen content is nevertheless a factor influencing the results. In figure 1 the data bearing on this point have been brought together by plotting the anaerobic glycolysis against the glycogen content of the slices in the 6 media studied. The glycogen analyses are reported as percentages of the wet weight of the liver slices, the lower values having been obtained by fasting the animals for 24–48 hours. In Ringer-bicarbonate medium there is a tendency towards somewhat higher values of $Q_6^{N_2}$ at the higher glycogen levels, but this is not a very

ANAEROBIC GLYCOLYSIS IN RELATION TO LIVER GLYCOGEN

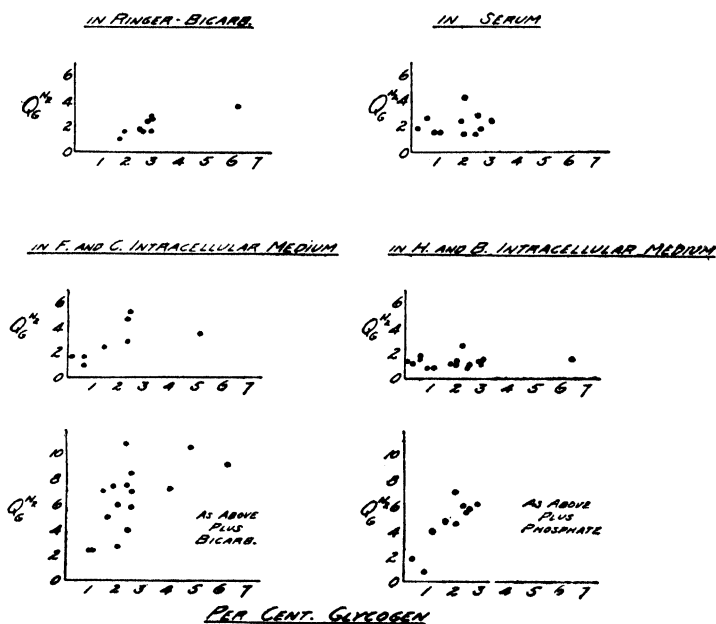


Fig. 1

conspicuous effect. Our results in this medium are accordingly more in line with those of Burk (1) than with the much higher values reported by Orr and Stickland (2). Glycolysis in serum is at about the same level as in Ringer-bicarbonate medium, though perhaps somewhat better maintained at the low glycogen levels. When the two intracellular media are compared, glycolysis is appreciably more rapid at the higher glycogen levels in the phosphate medium of Fuhrman and Crismon, while this effect is lacking in the bicarbonate medium of Hastings and Buchanan. We have noted above that glycolysis is most rapid in a medium containing high concentrations of potassium, phosphate and bicarbonate ions. These conditions may be obtained either by supplementing the Fuhrman and

Crismon medium with bicarbonate or by adding phosphate to the Hastings and Buchanan medium, as the lowermost graphs of figure 1 demonstrate. The two media modified in this way still differ in certain respects, notably in the absence of chloride in the former (cf. table 1), but it would appear that these differences have little if any influence on the level of anaerobic glycolysis.⁵

In each of the media described, the effect of added glucose has also been investigated, using livers from both fed and fasted animals. In 17 experiments, added glucose had no effect on anaerobic glycolysis which exceeded the limits of experimental error, nor was there any evidence that added glucose can be used anaerobically for glycogen synthesis.

Finally, it should be noted that the glycolytic rate is not constant with time in these special media, but usually decreases some 20–40 per cent during the 1-hour course of the experiment, as it does in the usual Ringer-bicarbonate medium (4, 6). The differences in glycolytic rate observed in the various media are consequently present from the beginning of the experimental period, and are not primarily due merely to a better maintenance of glycolysis under anaerobic conditions.

DISCUSSION. The rationale for employing an “intracellular” medium in tissue metabolism studies is explained by Hastings and Buchanan (7) as follows: Upon incubation of liver tissue *in vitro* in Ringer solution, there is a progressive change in the permeability of the cells with a resultant exchange of intracellular and extracellular ions. Placing the tissue in a medium similar to the intracellular fluid, it is proposed, maintains the intracellular environment in spite of exchange of ions across the cell boundary. Hastings pointed out (15) that such a medium could not be considered more physiological than an “extracellular” medium and would indeed be toxic to any organism into which it was injected, but their experiments nevertheless demonstrated that glycogen synthesis occurred in rat liver slices suspended in an intracellular but not in an extracellular medium. The conclusion was drawn that “the maintenance of a particular intracellular ionic environment is probably as important for the normal activity of certain intracellular enzymes as is the maintenance of a particular extracellular ionic environment for the maintenance of the normal activity of the cell as a whole”. The present authors concur in this point of view and our data further show that by adding phosphate to the Hastings intracellular medium the activity of the glycogenolytic enzymes is greatly enhanced, and the proportion of lactic acid formed is increased. This is not to say that such rapid glycogenolysis is a more normal state of affairs than that observed when the tissue is suspended in a more physiological medium such as serum; the import of the work is merely to describe conditions under which active glycogenolysis and lactic acid formation can be demonstrated. It is of interest, however, that Cherry and Crandall’s careful *in vivo* studies in dogs, using the London cannula technique, have emphasized the role of the liver in lactate formation under physiological conditions (16, 17).

⁵ Control experiments have also shown that the higher lactate formation in the intracellular as compared to the extracellular media is not due to the absence of calcium ions in the former.

As regards the mechanisms by which the three ions, phosphate, potassium and bicarbonate bring about their effects on anaerobic glycolysis, we have little information. Jeener (18) has recently demonstrated an accelerating effect of phosphate ions on aerobic glycogenolysis in rat liver, and attributes this to a facilitation of the phosphorylation of glycogen, the first step in glycogenolysis. This may well apply to the present experiments, but in addition it is necessary to account for the action of phosphate in favoring lactic acid over glucose formation (cf. table 3). The glucose is formed from hexose phosphates by phosphatase activity (19, 20) and it is possible that high phosphate concentrations in some way inhibit this step, although the reaction is not a reversible one in the usual sense. High phosphate concentrations may also maintain other phosphorylated intermediates in concentrations more optimal for glycolysis. The known effects of potassium and bicarbonate ions on isolated enzyme systems (21, 22) do not appear to clarify their rôle in enhancing anaerobic glycolysis, but it should be noted that Warburg long ago pointed out the accelerating effect of bicarbonate ions on glycolysis in tumors (23). The bicarbonate effect on glycolysis supplements, and may be a part of, its effect on respiration previously noted (29).

The lack of utilization of glucose for anaerobic glycolysis in rat liver slices in any of the media studied is of interest in contrast to its utilization for glycogen synthesis by the same tissue in Hastings and Buchanan's intracellular medium (7). The latter experiments were performed under aerobic conditions, and it has been shown that oxidative energy is necessary for maintenance of glycogen synthesis in liver slices (20, 24) and for uptake of inorganic phosphate by liver brei (25). That glucose is not utilized for anaerobic glycolysis in a medium in which it is utilized for aerobic glycogen synthesis may consequently be attributed to the need of oxidative energy (probably in the form of adequate concentrations of ATP) to bring about the initial phosphorylation of glucose required for its introduction into the glycolytic chain of reactions. But it should be noted that this requirement on the part of the liver is in contrast to that in most other tissues (23, 26-28) in which the rate of anaerobic glycolysis is accelerated by the presence of glucose in the medium. Finally, reference should be made to the suggestion of Fuhrman and Crismon (8) that the superiority of their intracellular medium in permitting improved recovery of the liver from exposure to anaerobic conditions is attributable to enhanced anaerobic glucose utilization. This is not supported by the present data, for the augmented glycolysis in this medium (which they also observed) does not involve utilization of added glucose.

SUMMARY AND CONCLUSIONS

1. The rate of anaerobic glycogenolysis in rat liver slices and the distribution of the end-products between lactic acid and reducing sugar have been investigated in various media.
2. Both the rate of glycogenolysis and the proportion of lactic acid formed are maximal in media containing high concentrations of potassium, phosphate and bicarbonate ions.
3. These effects are quite distinct from the influence on glycolysis of the initial

glycogen content of the liver slices. However, the relationship between glycogen content and rate of glycolysis is most clearly seen in the medium just described.

4. In the media supporting more active glycolysis, the formation of reducing sugar is also increased but to a much smaller extent than lactate formation; the dual pathway of glycogenolysis is consequently shifted in the direction of enhanced glycolysis.

5. The addition of glucose is without appreciable effect in any of the media studied; the conclusion of Orr and Stickland that normal rat liver *in vitro* (unlike liver tumors) exhibits anaerobic glycolysis but not glucolysis is to this extent confirmed and extended to conditions under which glycolysis is greatly augmented.

6. Certain other implications of these findings are discussed.

REFERENCES

- (1) BURK, D. A symposium on respiratory enzymes. University of Wisconsin Press, p. 235, 1942.
- (2) ORR, J. W. AND L. H. STICKLAND. *Biochem. J.* **35**: 479, 1941.
- (3) STICKLAND, L. H. *Ibid.* **35**: 859, 1941.
- (4) ROSENTHAL, O. AND A. LASNITZSKI. *Biochem. Ztschr.* **196**: 340, 1928.
- (5) ROSENTHAL, O. *Biochem. Ztschr.* **227**: 354, 1930.
- (6) DRUCKREY, H. *Arch. exper. Path. u. Pharmacol.* **180**: 231, 1935.
- (7) HASTINGS, A. B. AND J. M. BUCHANAN. *Proc. Nat. Acad. Sci.* **28**: 478, 1942.
- (8) FUHRMAN, F. A. AND J. M. CRISMON. *J. Biol. Chem.* **152**: 213, 1944.
- (9) BARKER, S. B. AND W. H. SUMMERSON. *Ibid.* **138**: 535, 1941.
- (10) GOOD, C. A., H. KRAMER AND M. SOMOGYI. *Ibid.* **100**: 485, 1933.
- (11) BENEDICT, S. R. *Ibid.* **92**: 141, 1931.
- (12) FOLIN, O. AND H. MALMROS. *Ibid.* **83**: 115, 1929.
- (13) CRAIG, F. N. *Ibid.* **150**: 209, 1943.
- (14) UMBREIT, W. H., R. H. BURRIS AND J. F. STAUFFER. *Manometric techniques and related methods for the study of tissue metabolism.* Burgess Publishing Co., Minneapolis, 1945, p. 164.
- (15) HASTINGS, A. B. *Harvey Lectures* **36**: 91, 1940.
- (16) CHERRY, I. S. AND L. A. CRANDALL, JR. *This Journal* **120**: 52, 1937.
- (17) CHERRY, I. S. AND L. A. CRANDALL, JR. *Ibid.* **125**: 41, 1939.
- (18) JEENER, R. *Arch. Internat. de Physiol.* **53**: 158, 1943.
- (19) CORI, G. T., C. F. CORI AND G. SCHMIDT. *J. Biol. Chem.* **129**: 629, 1939.
- (20) OSTERN, P., D. HERBERT AND E. HOLMES. *Biochem. J.* **33**: 1858, 1939.
- (21) BOYER, P. D., H. A. LARDY AND P. H. PHILLIPS. *J. Biol. Chem.* **146**: 673, 1942; *Ibid.* **149**: 529, 1943.
- (22) GREENSTEIN, J. AND H. W. CHALKEY. *J. Nat. Cancer Inst.* **6**: 143, 1945.
- (23) WARBURG, O., K. POSENER AND E. NEGELEIN. *Biochem. Ztschr.* **152**: 309, 1924.
- (24) CRANDALL, D. I. *J. Biol. Chem.* **160**: 343, 1945.
- (25) KALCKAR, H. *Enzymologia* **2**: 47, 1937.
- (26) ASHFORD, C. A. *Biochem. J.* **27**: 903, 1933.
- (27) SHORR, E. *Cold Spring Harbor Symposium on Quant. Biol.* **7**: 323, 1939.
- (28) WARREN, C. O. Unpublished observations on bone marrow anaerobic glycolysis.
- (29) WARREN, C. O. *J. Biol. Chem.* **156**: 559, 1944.

THE VALIDITY OF INTERNAL JUGULAR VENOUS BLOOD IN STUDIES OF CEREBRAL METABOLISM AND BLOOD FLOW IN MAN¹

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Since the introduction of a technique for internal jugular vein puncture in man (1), blood from this source has been assumed to be representative of mixed cerebral venous blood and has been utilized in numerous studies of cerebral blood flow and metabolism. The generally recognized fact that the torcular in man is often incomplete has not been considered a sufficient reason to invalidate this assumption (2). Recently, however, Batson (3) has presented anatomical evidence that an intercommunication exists between the cerebral, cranial, and extracranial venous circulation through the venous plexuses of the calvarium, but no data are available as to whether these are of sufficient magnitude to contribute significantly to internal jugular blood. Thus the possibility exists that the internal jugular vein may be contributed to in a variable manner on the two sides depending not only on variations in the distribution of the great venous sinuses, but also on the magnitude and distribution of the extracranial communications.

Physiologic verification of these anatomical studies are necessary. The fact that the intracranial and extracranial circulations often respond differently to hyperventilation provides a means of examining this point (4). Previous studies, using a method which is not dependent on the composition of internal jugular blood (5), have established that cerebral blood flow decreases during hyperventilation. At the same time flushing of the face is a frequent finding during hyperventilation, indicating an increase in the extracranial circulation. Thus it may be anticipated that in the presence of communication between intra and extracranial circulations shunting may take place from the increased extracranial circulation to the decreased intracranial circulation, thereby increasing the proportion of extracranial blood in the internal jugular vein. Simultaneous study of the blood from both internal jugular veins during hyperventilation may then demonstrate *a*, whether there is a difference in such dilution on the two sides, and *b*, whether there is a difference in the distribution of the great venous sinuses. Also, by comparing the composition of internal jugular blood as it leaves the skull with that from the lower third of the internal jugular vein and from the external jugular vein, the effects of addition of extracranial blood to internal jugular blood can be visualized.

¹ The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Cincinnati.

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METHODS. Co-operative patients without unilateral organic brain disease and who hyperventilated voluntarily were utilized. Blood drawn simultaneously from both internal jugular veins (at the jugular bulbs; hereafter, called bulb level internal jugular blood), was studied 21 times; from the bulb level and from the lower one-third of the same internal jugular vein, 5 times; and from external and bulb level internal jugular veins twice. Obturated needles were placed in position and specimens were drawn simultaneously under paraffin oil and stored in mercury vessels for analysis of oxygen and carbon dioxide contents by the Van Slyke manometric technique. Duplicate analyses of oxygen checked within 0.2 volume per cent and of carbon dioxide within 0.5 volume per cent.

RESULTS. Two patterns, already described by Gibbs (6), were noted in bulb level internal jugular blood during hyperventilation. In Pattern I the oxygen content falls and remains low while the carbon dioxide content rises slightly and then falls. In Pattern II the oxygen content rises slightly or remains unchanged, while the carbon dioxide content falls steadily. As compared to Pattern I, Pattern II more closely approaches the characteristics of arterial blood.

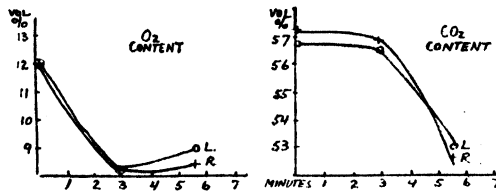
Of 21 studies of bilateral internal jugular blood at bulb level 7 (33 per cent) revealed Pattern I on both sides and the magnitude of the change was identical (fig. 1). On 14 occasions (67 per cent) there were differences between the two sides of more than 1 volume per cent in oxygen content and more than 2 volumes per cent in the carbon dioxide contents. Analysis of these 14 experiments revealed the following: 9 subjects showed Pattern I and one subject showed Pattern II on both sides, but significant differences between the magnitude of the changes were demonstrable on the two sides (fig. 2); 3 subjects showed Pattern I on one side and Pattern II on the other (fig. 3); 7 subjects differed significantly in the pre-hyperventilation control. Pattern II occurred 4 times on the left side and twice on the right.

Blood was drawn simultaneously from bulb level and from the lower third of internal jugular vein in 5 subjects. The blood from the bulb level revealed Pattern I in all instances, while blood from the lower third yielded Pattern II in 3 instances and Pattern I (but with higher oxygen content and lower carbon dioxide content) in two instances (fig. 4). External jugular blood yielded Pattern II in 2 experiments in which Pattern I was found simultaneously in bulb level blood (fig. 5). The pre-hyperventilation values were higher for oxygen and lower for carbon dioxide (i.e., more arterial) in the lower internal jugular and the external jugular blood as compared to bulb level internal jugular blood.

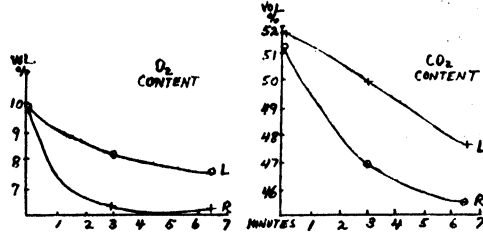
COMMENT. These results demonstrate that the composition of blood reaching both internal jugular veins at the level of jugular bulb differed significantly during hyperventilation in two-thirds of the subjects studied. In one-third differences were apparent in the resting values. These results can be explained only by assuming that the two internal jugular veins are usually contributed to by different sources of venous blood.

Gibbs, Lennox and Gibbs (6) studied blood drawn simultaneously from both internal jugular veins in 25 patients in the resting state. In eight of these there was a difference of more than 1.0 volume per cent between the two sides. Four

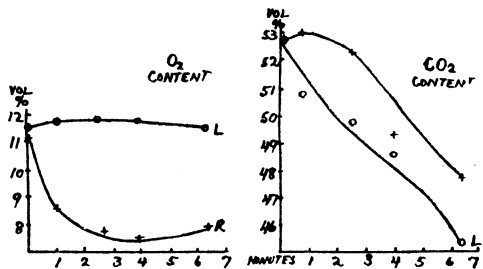
1 GRE. M-46 LEFT + RIGHT INTERNAL JUGULAR BLOOD



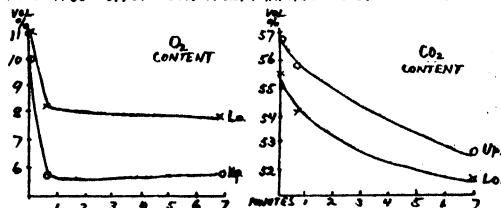
2 LBY. M-44 LEFT + RIGHT INTERNAL JUGULAR BLOOD



3 FER. M-40 LEFT + RIGHT INTERNAL JUGULAR BLOOD



4. HEM. M-60 UPPER + LOWER LEFT INTERNAL JUGULAR BLOOD



5. STR. M-61 LEFT INTERNAL + EXTERNAL JUGULAR BLOOD

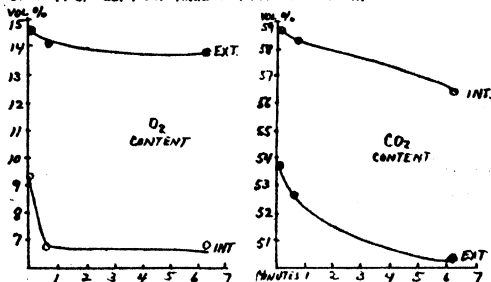


Fig. 1 Left and right internal jugular (at bulb); Pattern I and identical behavior on both sides.

Fig. 2 Left and right internal jugular (at bulb); Pattern I, but different degree of change on the two sides.

Fig. 3 Left and right internal jugular (at bulb); Pattern I on right, Pattern II on left.

Fig. 4 Internal jugular blood at bulb level and from lower third (left).

Fig. 5 External and internal jugular blood at bulb (left).

of these patients were presumed to have unilateral organic brain disease. Seven patients were hyperventilated and three showed a discrepancy in O_2 content of venous blood on the two sides at the end of hyperventilation. In spite of these findings the authors conclude that "in normal persons without evidence of unilateral cerebral damage, a sample from either internal jugular vein can be used as evidence of total venous drainage from the brain, at least so far as the oxygen utilization is concerned."

York, Hamburger and Himwich (7) compared internal jugular blood drawn "in rapid succession" from the two sides in 40 patients and found a difference in O_2 content of greater than 1.0 volume per cent in 7 patients. They concluded that the similarity in O_2 contents on the two sides in the quietly resting subject could be explained by assuming that the vascularity of any given cerebral region is related to the rate at which oxidations take place. They did not assume that the two internal jugular veins necessarily drain symmetric portions of the brain. Contributions from non-cerebral sources, however, were not considered.

The most common finding during hyperventilation was for the oxygen content to fall (Pattern I). Thus, of 49 studies of bulb level internal jugular blood, 42 revealed Pattern I. In external jugular blood, on the other hand, Pattern II (maintained or rising oxygen content) prevailed and when blood from the lower third of the internal jugular vein was compared to that from bulb level, a shift toward Pattern II was noted in all cases. Thus, the addition of blood from extracranial sources to internal jugular blood leads to a shift from Pattern I toward Pattern II.

The fact that the oxygen content of internal jugular venous blood fails to fall in some instances (Pattern II) was interpreted by Gibbs to indicate feeble vasoconstriction in such individuals (8). Our data do not support such a conclusion but suggest that blood reaching the jugular bulbs may be contributed to on the two sides in a variable manner. Batson's studies indicate that anastomoses between the intra and extracranial circulations may be the source of such variations. While the magnitude of the communications between the two circulations is unknown, the occurrence of subjects with Pattern I on one side and Pattern II on the other, suggests that in some instances the proportion of extracranial blood reaching one internal jugular vein may be relatively large. Until the size of this intercommunication is known, it is not possible to assume that internal jugular blood is representative of mixed cerebral venous blood alone, even when the findings on the two sides are identical. The latter only justifies the assumption that in such subjects the contributions from extracranial sources reaching the two lateral sinuses are equal, but does not reveal their size. Since Pattern I was the most common finding in bulb level blood, while blood containing larger proportions of extracranial blood (low internal jugular and external jugular) regularly becomes more arterial (Pattern II), it may be concluded that Pattern I most closely approximates true cerebral blood during hyperventilation, although it still may include an unknown proportion of extracranial blood.

These observations preclude the use of internal jugular blood in quantitating cerebral blood flow or metabolism and suggest the precautions which must be

taken in evaluating such studies. Recent methods of estimating cerebral blood flow by means of clearance of inert gases from jugular blood (2) would appear to be valueless unless it can be demonstrated that the blood gas behavior falls into Pattern I during hyperventilation. Even then, the results are only of qualitative or comparative value and have no significance in a quantitative sense.

Thus, it is concluded that internal jugular venous blood in man, obtained by the traditional technique, cannot be considered as representative of mixed cerebral venous blood and that therefore it is of limited value in the study of cerebral blood flow or metabolism in man. In any one individual it is impossible to predict the anatomical arrangement of the venous circulation or the magnitude of extracranial communications so that bilateral studies are always necessary.

REFERENCES

- (1) MYERSON, A., R. D. HALLORAN AND H. D. HIRSCH. *Arch. Neurol and Psychiat.* 17: 807, 1927.
- (2) KETY, S. S. AND C. F. SCHMIDT. *This Journal* 143: 53, 1945.
- (3) BATSON, O. V. *Federation Proc.* 3: 139, 1944.
- (4) ENGEL, G. L., E. B. FERRIS, S. RAPOPORT, M. LOGAN AND C. D. STEVENS. To be published.
- (5) FERRIS, E. B. *Arch. Neurol and Psychiat.* 46: 377, 1941.
- (6) GIBBS, E. L., W. G. LENNOX AND F. A. GIBBS. *Am. J. Psychiat.* 102: 184, 1945.
- (7) YORK, G. E., E. HOMBURGER AND H. E. HIMWICH. *Arch. Neurol. and Psychiat.* 55: 578, 1946.
- (8) GIBBS, E. L., F. A. GIBBS, W. G. LENNOX AND L. F. NIMS. *Arch. Neurol and Psychiat.* 47: 879, 1942.

THE RELATIONSHIP OF THE PARATHYROID GLANDS TO THE ACTION OF ESTROGEN ON BONE

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The administration of large doses of estrogen to young rats for a limited period of time causes a profound increase in the density of trabeculation in the sub-epiphyseal region of certain long bones (1, 2, 3), this alteration sometimes being termed "hyperossification". Since this effect cannot be elicited in hypophysectomized rats (4) and since a somewhat similar histological modification has been induced under varied experimental conditions by the administration of parathyroid hormone to young rats (5, 6, 7, 8), it might be postulated that this phase of the action of estrogen on bone is mediated through the anterior hypophysis and the parathyroid glands. In fact, Kibrick et al. (4, p. 94) have stated: "It may be . . . that the hypophysis influences calcium metabolism and perhaps skeletal histology through the parathyroid glands . . ."

Our interest in this problem stems from the fact that such a hypothesis implies regulatory control of the parathyroid glands by the anterior hypophysis. The belief that the hypophysis secretes a parathyreotropic factor arose primarily from the demonstration by numerous investigators that the administration of anterior hypophyseal extracts to a variety of experimental animals causes cellular enlargement in the parathyroid glands. Evidence regarding changes in the parathyroid glands following hypophysectomy is controversial, and since significant cytological changes were not observed in them following hypophysectomy in the monkey, the importance of hypophyseal control over these glands was questioned (9). Additional support for this position was supplied subsequently by Carnes, Osebold and Stoerck (10) who demonstrated that the parathyroid glands of young hypophysectomized rats were able to maintain normal serum calcium and inorganic phosphorus concentrations even when these animals were subjected to the stress of a low calcium diet. Hence, we were led to suspect that the parathyroid glands do not play an essential rôle in the action of estrogen on bone. This belief is borne out by the following experiments which show that the postulation of the secretion of a parathyreotropic factor by the anterior hypophysis is not necessary to explain the histological modification of bone elicited by estrogenic treatment.

METHODS. The study was carried out on fifty-two growing female rats of the Long-Evans strain which ranged from 32–90 days of age at the beginning of the experiment. The members of each litter were divided into the following groups: non-operated, oil-treated; non-operated, estrogen-treated; parathyroidectomized, oil-treated; and parathyroidectomized, estrogen-treated. The injection of estrogen (alpha-estradiol dipropionate¹ in peanut oil) was begun the day after para-

¹ We wish to thank Dr. F. E. Houghton of Ciba Pharmaceutical Products, Inc., for supplying generous quantities of alpha-estradiol dipropionate (Di-Ovocylin).

thyroidectomy, 250 μ g. (0.05 cc. solution) being injected subcutaneously on alternate days for twenty days. The oil-treated animals received comparable amounts of peanut oil. In performing the parathyroidectomies as much thyroid tissue was left as possible. Completeness of removal of the parathyroid glands was verified by microscopic examination of serial sections of mid-cervical structures extending from the hyoid bone into the superior mediastinum. If parathyroid tissue was found the animal was eliminated from the parathyroidectomized group. The tibias were weighed at autopsy and the proximal ends prepared for histological study. Thickness of the epiphyseal cartilage was determined by taking the mean of three measurements, one being made at the middle and the others about midway from the middle to either side of the epiphyseal cartilage.

RESULTS. *Effect of estrogen treatment on intact rats.* During the period of the experiment, intact, estrogen-treated rats showed an average gain of 32 per cent in body weight as contrasted to a 54 per cent gain by the controls (table 1). By

TABLE I
Effect of estrogen on body weight and epiphyseal cartilage

TREATMENT	NO. RATS	PER CENT GAIN IN BODY WT.	RATIO FEMUR (MG.M.)/ BODY WT. (GM.)	EPIPHYSEAL CARTILAGE		
				Mean thickness (mm.)	Mean no. cells* per row	Mean no. hyper- cells
Intact, oil-treated	12	54	5.1	0.304 (0.224-0.384)	21.1 (16-25)	3.9
Intact, est.-treated	12	32	5.5	0.187 (0.176-0.208)	13.1 (11-18)	3.2
Parathy., oil-treated	10	25	4.5	0.304 (0.256-0.352)	20.0 (16-22)	3.0
Parathy., est.-treated	14	17	5.6	0.205 (0.128-0.320)	14.7 (11-23)	3.2

* Exclusive of hypertrophied cells.

absolute weight the femurs of the treated rats were not heavier than those of their controls, although the ratio of femur weight to body weight was greater.

Estrogen modified the structure of two portions of the growing bone, namely, the epiphyseal cartilage and the sub-chondral region of ossification. The epiphyseal cartilage suffered a 38 per cent reduction in thickness due primarily to an inhibition of the proliferative activity of the cartilage cells as indicated by the significant reduction in the number of cells in the cellular columns of the cartilage (table 1). These columns showed a small degree of disorientation. Significant structural evidence of interference with the resorption of cartilage was not observed, the average number of cells in the rows of hypertrophied cartilage cells not being altered (table 1) and the blood capillaries of the bone marrow appearing to invade the enlarged cartilage lacunae in a normal manner. In general, these observations support the findings of other workers (2, 11).

In the sub-epiphyseal region there appeared, following treatment with estrogen, an increase in the density of thick, anastomotic bony trabeculae (figs. 1, 2)

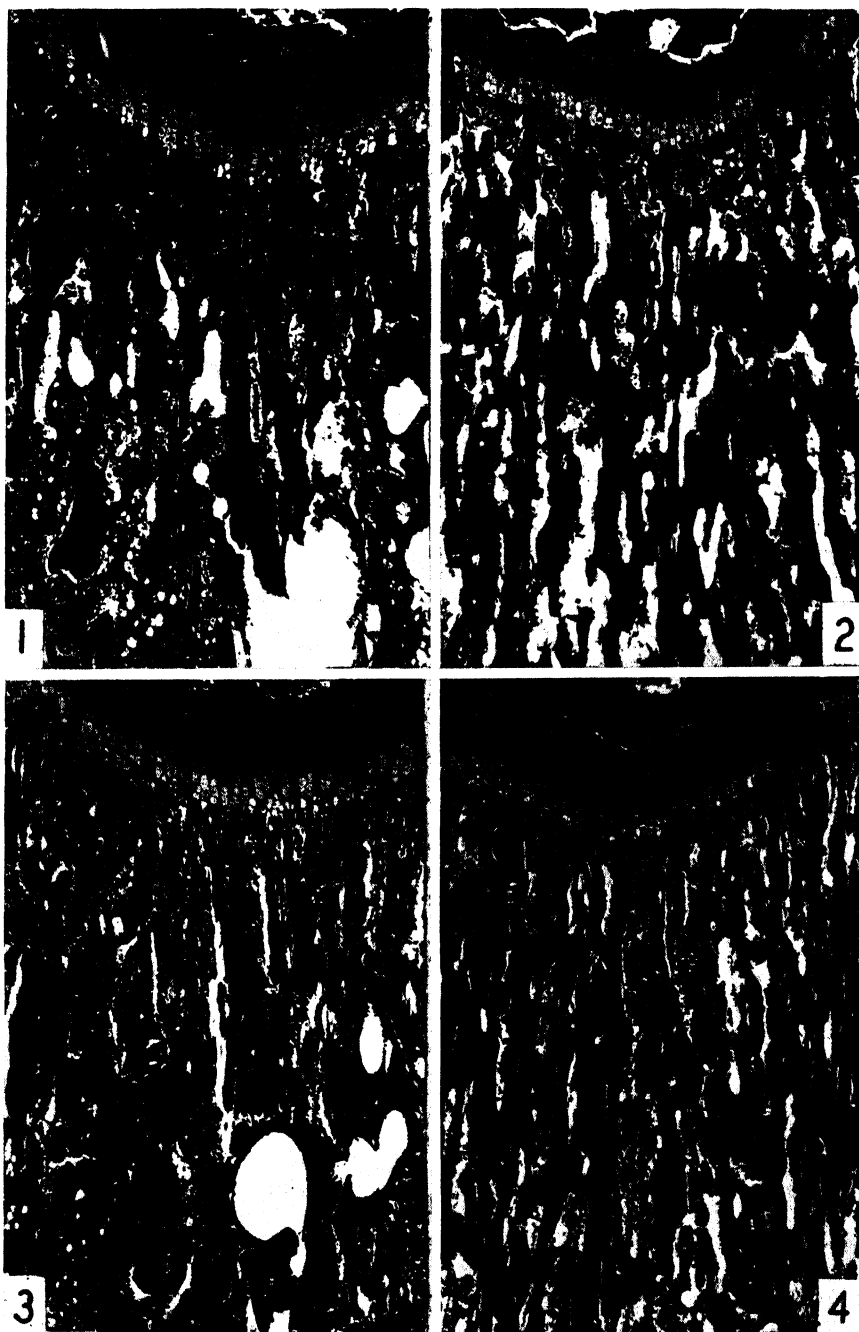


Fig. 1. Intact, oil-treated.

Fig. 2. Intact, estrogen-treated.

Fig. 3. Parathyroidectomized, oil-treated.

Fig. 4. Parathyroidectomized, estrogen-treated.

The figures illustrate the proximal ends of the tibias taken from litter-mate animals.

which was so marked that a dense band of sub-chondral bone was macroscopically visible in the split tibias prior to fixation. These dense trabeculae extended farther into the diaphysis than in the controls. Continued bone formation was indicated by the presence of osetoblasts immediately beneath the epiphyseal cartilage. However, towards the diaphysis, both osteoblasts and osteoclasts were reduced in number, suggesting that here bone was neither being actively formed nor resorbed.

Effect of estrogen in parathyroidectomized rats. Parathyroidectomy alone caused an inhibition of gain in body weight, these rats showing an average increase of 25 per cent as compared with a 54 per cent gain by the intact, oil-treated animals. The 17 per cent gain of the estrogen-treated, parathyroidectomized rats was still less (table 1). In these treated animals the ratio of femur weight to body weight was elevated being 5.6 as compared with 4.5 for the oil-treated, parathyroidectomized controls.

Over the twenty-one day period of the experiment, parathyroidectomy alone caused no detectable histological changes (figs. 1, 3). The anatomical modifications induced by estrogenic treatment were similar to those observed in the intact animals. Thus, the reduction in thickness of the epiphyseal cartilage and in the number of cells in the cartilage cell columns was of similar magnitude; likewise, there was no difference in the number of cells in the rows of hypertrophied cartilage cells (table 1). The histological alteration of the sub-epiphyseal region was similar to that described for intact rats (figs. 3, 4) except that in a few instances it was less marked.

DISCUSSION. These results show that the inhibition of the proliferative activity of the epiphyseal cartilage and the induction of increased sub-epiphyseal trabeculation by estrogen is carried out by some means other than stimulation of the parathyroid glands because estradiol was about equally effective in both intact and parathyroidectomized rats. It is, therefore, unnecessary to invoke the secretion of a parathyreotropic factor by the anterior hypophysis. Since one of the major functions of the growth hormone is stimulation of proliferation of the epiphyseal cartilage cells (12), the reduction in divisional activity of cartilage cells resulting from estrogen treatment may be explained on the basis of either the suppression of secretion of the growth hormone by the anterior hypophysis or by neutralization of the action of this factor at the epiphyseal cartilage. It is probable also that a partial inanition resulting from the toxicity of the high doses of hormone administered also contributed to the inhibition of growth in the epiphyseal cartilage. We did not include pair-fed controls but Day and Folliis (2) have demonstrated that restricted food intake results in changes in the cartilage which are similar to, but less marked, than those following intensive estrogen treatment. The precise manner in which estrogen acts on bones of the rat and the possible involvement of ductless glands other than the parathyroids cannot be elucidated at this time. Our histological observations support the conclusions of others (2, 3) that the trabeculae accumulate because of defective bone resorption. The somewhat less marked response observed in some of our parathyroidectomized rats as compared with their intact, estrogen-treated con-

trols, may have resulted from unavoidable damage inflicted upon the thyroid during removal of the parathyroid glands.

Finally, one additional point requires consideration. There is considerable doubt as to what extent the ratios of femur weight to body weight may be taken as an indication of bone formation. An elevated ratio might just as well indicate a reduction in weight of the soft tissues of the body, in fact, diesters of estradiol have been shown to cause a decrease in the deposition of fat (13).

SUMMARY

Study of the tibias of parathyroidectomized and intact rats treated with large doses of alpha-estradiol dipropionate showed that "hyperossification" could be induced in the absence of the parathyroid glands. It was concluded that even though the hypophysis is essential to this phenomenon, the parathyroids are unnecessary and, therefore, the secretion of a parathyreotropic factor by the hypophysis need not be postulated.

REFERENCES

- (1) SIMPSON, M. E., E. A. KIBRICK, H. BECKS AND H. M. EVANS. *Endocrinology* **30**: 286, 1942.
- (2) DAY, H. G. AND R. H. FOLLIS. *Endocrinology* **28**: 83, 1941.
- (3) SILBERBERG, M. AND R. SILBERBERG. *Am. J. Anat.* **69**: 295, 1941.
- (4) KIBRICK, E. A., M. E. SIMPSON, H. BECKS AND H. M. EVANS. *Endocrinology* **31**: 93, 1942.
- (5) BAUER, W., J. C. AUB AND F. ALBRIGHT. *J. Exper. Med.* **49**: 145, 1929.
- (6) SELYE, H. *Endocrinology* **16**: 547, 1932.
- (7) SHELLING, D. H., D. E. ASHER AND D. A. JACKSON. *Bull. Johns Hopkins Hosp.* **53**: 348, 1933.
- (8) BURROWS, R. B. *Am. J. Anat.* **62**: 237, 1937.
- (9) BAKER, B. L. *Anat. Rec.* **83**: 47, 1942.
- (10) CARNES, W. H., J. OSEBOLD AND H. C. STOERCK. *This Journal* **139**: 188, 1943.
- (11) LIPPMAN, H. N. AND J. B. DE C. M. SAUNDERS. *J. Endocrinology* **3**: 370, 1944.
- (12) RAY, R. D., H. M. EVANS AND H. BECKS. *Am. J. Path.* **17**: 509, 1941.
- (13) KORENCHESKY, V., R. BURBANK AND K. HALL. *Biochem. J.* **33**: 366, 1939.

THE EFFECT OF DINITROPHENOL ON THE OXYGEN CONSUMPTION OF ALBINO MICE AT GRADED LEVELS OF ENVIRONMENTAL TEMPERATURE¹

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The hyperthermia which follows administration of 2,4-dinitrophenol to mammals is doubtless a consequence of an augmented rate of tissue metabolism produced by the drug. It has been reported by several investigators (Magne, Mayer and Plantefol, 1932; Giaja and Dimitrijevic, 1933; Tainter, 1934; Riddle and Smith, 1935; Zummo, 1935) that the hyperthermia and heightened metabolic rate are much less evident at low environmental temperatures than at about thermal neutrality.

The fatal dose of dinitrophenol in mice varies with the environmental temperature (G. J. Fuhrman *et al.*, 1943). Previous investigators have often used a dose which proved fatal at the higher environmental temperatures; body temperature and metabolic measurements on these moribund animals are of questionable value. In order to study a wide temperature range, a dose of the drug which is tolerated at all experimental temperatures must be used. This has been done in the present investigation.

METHODS. Oxygen consumption was measured at environmental temperatures of 30°, 25°, 20°, 15° and 10°C. by means of a manometric apparatus already described (G. J. Fuhrman *et al.*, 1946). The water thermostats could be regulated to $\pm 0.1^\circ\text{C}$. Sixteen albino mice, 6 months old, were used; 4 females and 12 males with an average weight of 25 grams. Each mouse served as its own control; that is, the oxygen consumption of each of the sixteen mice was determined twice at each environmental temperature, once injected with dinitrophenol and once as a control. "Paired" values were thus obtained which could be analyzed statistically with greater accuracy than values obtained on different animals. The time between experiments insured that no previous injections of dinitrophenol affected control determinations. Since it was found that the oxygen consumption rates of mice differed in the morning and afternoon (G. J. Fuhrman *et al.*, 1946), each mouse was always used at the same time of day. Oxygen consumption is expressed as a weight-specific rate here denoted by the symbol Q_{O_2} , which is defined as the milliliters of oxygen consumed, STP, per hour per gram of animal weight. Body temperature (rectal) measurements were made on some of the animals at the varied environmental temperatures using a Leeds and Northrup potentiometer with an iron-constantan thermocouple inserted to a depth of 15 mm.

Sodium 2-4 dinitrophenol (DNP) was dissolved in Ringer's solution and ad-

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ministered subcutaneously in the dorsal neck region in a dose of 15 mgm. per kgm. All injections were made at room temperature.

RESULTS AND DISCUSSION. In table 1 are recorded the means and standard errors of the differences for the oxygen consumption rates (Q_{O_2}) at different environmental temperatures for normal and dinitrophenol-injected mice. There is a linear relationship between environmental temperature and Q_{O_2} in control animals from 25°C. to 10°C. (fig. 1). Herrington (1940) has also shown such a linear relationship between temperature and metabolism in mice. However, in his group-determinations the relationship appears to obtain from 14° to 30°C. instead of only to 25°C.

After 70 minutes at each of the experimental temperatures used, the rectal temperatures of the control and DNP-injected mice (4 animals at each temperature) did not differ by more than 1°C. (cf. Scheff and Rabati, 1938).

The effect of 15 mgm. per kgm. DNP on observable activity (G. J. Fuhrman *et al.*, 1946) is a reduction of about 50 per cent below that of the controls at 30°

TABLE 1

TEMP.	NO. ANIMALS	Q_{O_2}	Q_{O_2} DNP	Q_{O_2} DNP - Q_{O_2}	SE	P_t	$-\log_e P$
(°C.)							
30	16	3.165	4.403	+1.238	0.222	0.0005	7.60
25	16	4.936	5.095	+0.159	0.246	0.263	1.01
20	16	5.662	5.906	+0.244	0.331	0.237	1.44
15	16	6.496	6.704	+0.209	0.181	0.134	2.01
10	8	7.305	7.510	+0.205	0.250	0.213	1.54
							13.60

$$\chi^2 = 27.2; \text{ with 10 degrees of freedom } 0.01 > P_{\chi^2} > 0.001.$$

and 25°C. and a progressive decrease in activity of the controls and increase in that of the DNP-injected animals at 20° and 15°C. until they exhibit about equal activity at 10°C.

The effect of DNP on metabolism at varied temperatures is shown graphically in figure 1. Since the individual mice were used as their own controls, the method of paired differences gives accurate and sensitive results. With this method there is a significant difference between Q_{O_2} of controls and Q_{O_2} of DNP-injected mice only at 30°C. However, at all other temperatures the mean Q_{O_2} of DNP-injected mice is higher than that of controls. Although only one difference is significant, their combination according to the method of Fisher (1936, paragraph 21.1) gives a total probability of 0.005, indicating that the differences observed in the series as a whole could not have occurred by chance.

A series of observations in the literature indicates that DNP is a more potent calorogenic agent as the *body* temperature is reduced. This is true for anesthetized cats in which chemical cold-defense mechanisms are inactive (Hall, Crismon and Chamberlin, 1937). Our data do not permit a separation of the calorogenic

action of DNP and its effect on the activity of chemical cold-defense mechanisms. As environmental temperature is reduced, the control mice show increasing Q_{O_2} , due, in these acute experiments, to active chemical defense in maintaining body temperature. The animals given DNP show less observable activity than the control mice. Presumably chemical defense and body activity contribute more to the total Q_{O_2} of control animals than in the DNP-injected mice, since the calorigenic action of the DNP contributes to the maintenance of body temperature. However, since the body temperature of the mice given DNP in this study was not lower than that of the controls even at 10°C., there is

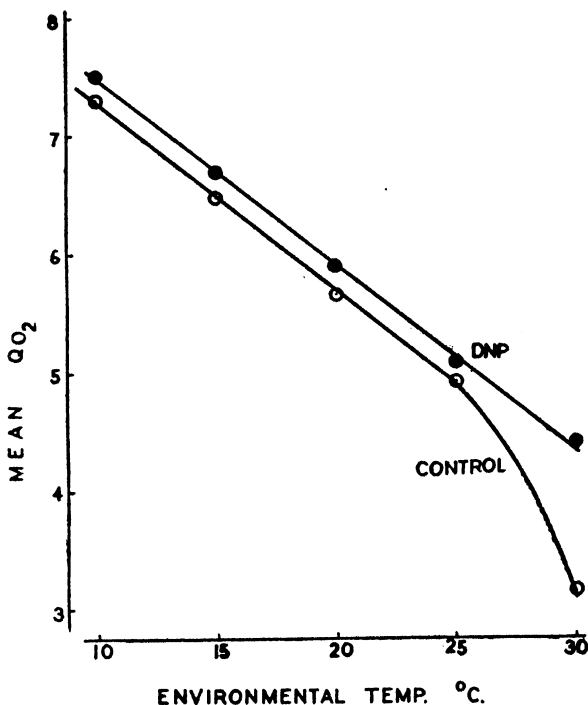


Fig. 1. Metabolic rates of normal and dinitrophenol-injected mice at varied environmental temperatures.

no evidence that chemical defense has been rendered incompetent to maintain body temperature, as was reported by Tainter (1934).

It is suggested that the dosage of DNP required to render the chemical defense mechanisms inadequate during exposure to cold would, in the mouse, have to exceed 15 mgm. per kgm., an impractical procedure where the mice are required to survive administration of the drug.

SUMMARY

1. Oxygen consumption of normal and dinitrophenol-injected mice was measured manometrically at environmental temperatures of 30°, 25°, 20°, 15° and 10°C.

2. Dinitrophenol, 15 mgm. per kgm. subcutaneously, augmented the metabolic rate of mice at an environmental temperature of 30°C., but did not significantly increase it above controls at the other temperatures studied. At these lower temperatures the metabolic rate of both control and dinitrophenol-injected mice was higher than that of control animals at 30°C.

3. Body temperature maintenance in control and dinitrophenol-injected animals is discussed.

REFERENCES

- FISHER, R. A. Statistical methods for research workers. Oliver and Boyd, London, 1936.
- FUHRMAN, G. J., F. W. WEYMOUTH AND J. FIELD, 2D. J. Pharmacol. and Exper. Therap. **79**: 176, 1943.
- FUHRMAN, G. J., E. D. McLIN AND M. L. TURNER. This Journal, in press, 1946.
- GIAJA, J. AND I. N. DIMITRIJEVIC. Arch. internat. pharmacodyn. et therap. **45**: 342, 1933.
- HALL, V. E., J. M. CRISMON AND P. E. CHAMBERLIN. J. Pharmacol. and Exper. Therap. **59**: 193, 1937.
- HERRINGTON, L. P. This Journal **129**: 123, 1940.
- MAGNE, H., A. MAYER AND L. PLANTEFOL. Ann. Physiol. Physiocochem. biol. **8**: 1, 1932.
- RIDDLE, O. AND G. C. SMITH. J. Pharmacol. and Exper. Therap. **55**: 173, 1935.
- SCHEFF, G. AND F. RABATI. Biochem. Ztschr. **298**: 101, 1938.
- TAINTER, M. L. J. Pharmacol. and Exper. Therap. **51**: 45, 1934.
- ZUMMO, C. Arch. internat. pharmacodyn. et therap. **51**: 450, 1935.

TOLERANCE OF ADULT CHICKENS TO HYPOTHERMIA¹

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Baldwin and Kendeigh (1) reported that the lower lethal body-temperature in the adult house wren and possibly other passeriform species is approximately 21.7°C., whereas in the nestling wren, before the temperature control mechanism has been developed, the lethal temperature is about 7°C. Randall (2) who exposed 7-day-old chicks to an air temperature of 10°C. found that body temperature dropped within 3 hours to 15°C., at which point respiration ceased and death soon ensued unless pure oxygen was administered. He concluded that hypothermic death is caused primarily by anoxic paralysis of the respiratory mechanism.

Randall (3) has studied the effects of alternate chilling and warming in the adult chicken, but to the writer's knowledge no one has determined the lower lethal body-temperature or has studied the physiological effects of prolonged hypothermia in the mature fowl. The report which follows concerns such a study.

METHODS. Hypothermia was induced in adult White Leghorn cocks and hens, approximately 1 year of age, by suspending them, up to the neck, in water of the desired temperature. Activity of the birds in the bath was prevented by placing them in cloth jackets, which immobilized the wings, and by tying the feet. Rectal temperatures were taken at a depth of 7.5 cm. All of the experiments were conducted at room temperature. Details of the treatments are shown in the tables.

RESULTS. *Tolerance of cocks and hens to severe hypothermia.* The cocks and hens were placed in water ranging in temperature from 6 to 11.7°C. and maintained until respiration ceased. The details of the treatments and results are shown in table 1. The lethal body-temperatures of three hens suspended in water at 6°C. were 23.3, 23.3 and 23.9°C., and their survival periods were 50, 50 and 65 minutes, respectively (hens 1, 2 and 3). The lethal temperatures of five hens exposed to water-bath temperatures of 9 to 11.7°C. were 22.8, 23.3, 23.6, 22.8 and 23.6°C., and their periods of survival were 83, 65, 90, 90 and 79 minutes, respectively. Thus, there was little difference in the lethal temperatures of hens receiving the different treatments, but the periods of survival were, in most hens, directly proportional to the temperature of the water.

The lethal temperatures of the four males, 19.4, 22.2, 21.1 and 20.0°C., were in every case lower than those for the hens. The time of survival, as in the hens, varied directly with the water-bath temperature.

¹ Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, Department of Poultry Husbandry.

TABLE 1
Tolerance of hens and cocks to severe hypothermia

HEN NO.	TEMPERATURE, °C., OF			TIME		HEART RATE	RESPIRATORY RATE
	Air	Water	Body of hen	minutes	minutes		
1	21	6	N† to 26.1 23.3	30 20	50	105	R. ceased
2	21	6	N to 26.1 23.3	30 20	50	110	R. ceased*
3	21	6	N to 27.8 23.9	30 35	65	130 78	** R. ceased
4	20.5	9	N to 31.7 22.8 22.8	30 50 3	83	220	24 5 R. ceased
5	20.5	9	N to 23.6 23.3	60 5	65		4 R. ceased
6	22	10	N to 33.3 25.0 23.6	30 40 20	90	200 88	27 6-8 R. ceased
7	21	10.5	N to 27.8 24.4 22.8	30 30 30	90	140 80	** R. ceased
8	20.5	11.7	N to 25.3 23.6	42 37	79	80	5 R. ceased
MALE NO.							
1	20	6	N to 30.0 23.3 19.4	15 20 15 15	65	18.5	15 8 5 R. ceased
2	20	7.2	N to 31.7 25 22.2	10 45 20	75	230	21 R. ceased
3	20	7.2	N to 25.6 21.1	30 47	77	92	6 R. ceased
4	20	10.0	N to 29.2 24.2 21.6 20.0	15 25 20 15 15	90	160 68	12 10 10 6 R. ceased

* Bird resuscitated by artificial respiration and by warming in water at 46°C.

** Warm collar around neck.

† Normal temperature of approximately 41.5°C.

Within 10 to 15 minutes after the birds were placed in the water baths, they were shivering violently. By the time body temperature had dropped to approximately 26°C., the intensity of shivering had decreased and continued to decrease, but did not cease until death. As the degree of shivering diminished, respiratory rate decreased and respiration became irregular and arrhythmic, and as the respiratory mechanism began to fail, cyanosis of head and comb developed. Heart rate continued to decline, and when respiration ceased the rates of three of the hens (nos. 3, 6 and 8) were 78, 88 and 80, respectively.

Tolerance of hens to moderate hypothermia. The tolerance of hens to moderate hypothermia (27.8 to 31.1°C.) was determined by placing the birds in water at temperatures ranging from 20 to 27.8°C. The results are shown in table 2.

The hens were first placed in the water at a temperature of about 24°C., and within 1 to 1½ hours, body temperature had dropped from a normal of approximately 41.5 to 31 or 32°C. The temperature of the water was then elevated to 25 to 27°C. for four of the birds. This change decreased the rate at which body temperature continued to fall. By changing the temperature of the water when necessary, it was possible to maintain body temperature within the range of 27.8° to 31.1°C. for most of the time.

During the last 5 to 10 hours of the treatments, when the temperature control mechanism had become almost exhausted, it was necessary to increase the water-bath temperature in order to maintain body temperature at the desired level. When the birds succumbed to the treatments, the water-bath temperature was, in most cases, only about 1°C. higher than the body temperature of the hen.

The periods of survival for the hens thus treated were 44, 42.0, 40.25, 36.5 and 35.0 hours, and their lethal body-temperatures were 27.8, 29.4, 27.8, 28.9 and 28.9°C., respectively.

These birds were given water to drink at frequent intervals during the treatments, and they had feed in their crops when the experiments were initiated. Since these birds, after becoming hypothermic, were in a state of torpor and were unable to ingest and assimilate food normally, 3 of them (nos. 11, 12 and 13) had small amounts (ca. 1 gram) of glucose administered to them intravenously or subcutaneously 3 or 4 times during the course of the experiments. The injections had no effect upon the tolerance of these birds to the hypothermia, since their periods of survival were no greater than those of the birds not injected. It is believed that hunger was not an important factor in the responses of the birds to the hypothermia.

It was during the first few hours of the treatments that shivering of the birds was most intense. Also the heart rate during the first part of the period was increased but later decreased. Later the respirations and respiratory movements became irregular and arrhythmic, characterized by shallow, gasping movements. These symptoms were then followed by cyanosis of head and comb, decreased respiratory rate and heart rate, and death soon ensued unless the birds were warmed. In these experiments when the birds began to show the symptoms described above, the temperature of the water in the bath was elevated (see table 2) and rubber collars, through which warm water circulated, were placed around the necks of the birds.

TABLE 2

Tolerance of hens to moderate hypothermia

HEN NO.	TEMPERATURE, °C., OF			TIME		HEART RATE	RESPIRATORY RATE
	Air	Water	Body of hen	hours	hours		
9	26.1 to 28.9	24	N† to 32.8	1.0			
		20	30	0.5	1.5	168	
		20	26.7	0.5	2.0	100	
		20	25.0	0.5	2.5	80	
		20	22.8	2.25	4.75		*
		21.1	24.4	2.25	7.00		
		26.7	28.3	1.00	8.00		
		25.6 to 26.7	31.1	15.0	23.00	170	18
		27.2	29.4	18.5	41.5	150	15
		27.2	27.8	2.5	44.0		R. ceased
10	23.9 to 27.2	24.4	N to 31.1	1.0		270	
		26.7	30.0	3.5	4.5	200	22
		26.7	27.8	13.0	17.5		10*
		27.2	28.9	12.0	29.5	120	12**
		27.2	27.8	7.75	37.25	120	12*
		27.8	29.4	4.5	41.75	130	10
		27.8		0.25	42.0		R. ceased
11	23.9 to 27.2	24.4	N to 31.1	1.25		280	*
		27.2	29.4	9.25	10.5	135	16
		27.2	28.9	13.00	23.5	170	20
		27.2	30.0	13.75	37.25	150	18
		27.8	27.8	3.00	40.25		R. ceased
12	23.9 to 27.2	24.4	N to 31.1	1.25		300	*
		27.2	31.1	2.25	3.50	200	25
		25.6	29.4	8.0	11.5	165	18
		26.7	28.9	13.0	24.5	105	14
		27.2	30.0	6.0	30.5	74	13
		27.2	28.9	5.5	36.0		8
		27.2	28.9	0.5	36.5		R. ceased
13	23.9 to 27.2	24.4	N to 32.2	1.25			
		25.0	28.3	2.00	3.25	180	22
		25.0	27.8	1.00	4.25	120	22
		22.2	26.1	1.25	5.50	100	8*
		25.6 to 27.8	31.1	7.00	12.50	136	
		26.1	29.4	10.50	23.00	160	12
		27.2	28.9	11.50	34.50		12
		26.7	28.9	0.50	35.00		R. ceased

* Warm collar around neck.

** Warm collar off neck.

† Same as in table 1.

It was found that warming the neck by this means improved respiration and increased the tolerance of the birds to the treatments, under certain conditions. When the birds were exposed to water-bath temperatures such as described in table 2, and others not reported herein, the warm collar was effective, but when the water temperature was much lower, as shown in table 1, the collar had little or no effect upon subsequent behavior of the bird.

Usually shivering decreased in intensity and virtually ceased in the hens after about 10 to 15 hours of treatment, and the birds behaved as though lightly anesthetized for the remainder of the time. Also, the respiratory rate and movements slowed and became more regular, but the rate remained fairly constant at 10 to 15 per minute in most cases, until just before death, where it usually dropped to from 6 to 8. Heart rates were taken at varying intervals up to 3 to 6 hours before death in most of the hens. The rates during most of this period varied from 120 to 170 for all hens except one which was 74, and body temperatures ranged from 27.8°C. to 31.1°C. In one hen, no. 10, the heart rate, which was taken $\frac{1}{4}$ hour before death, was 130 per minute. Since body temperature changed little, if at all, during the last stages of the survival period, it is likely that the heart rates of most hens when respiration ceased were at least 100 per minute, a rate considerably higher than that at death for birds exhibiting more severe hypothermia (see table 1).

DISCUSSION. The results of these, and other experiments, indicate that the critical body temperature of cocks and hens is about 25 to 27° C. When the body temperature reaches this level, the birds exhibit signs of respiratory failure, and body temperature continues to drop to the lethal limits of 22.8 to 23.9°C. for the hens and 19.4 to 22.2 for the cocks, unless the birds are warmed. If, however, body temperature is maintained above the critical level, at 28 to 30°C., hens can tolerate hypothermia of this magnitude from 35 to 44 hours.

In either case hypothermic death, as shown for chicks by Randall (2), is caused by paralysis of the respiratory mechanism. The temperature of the blood flowing to the head and respiratory center no doubt plays an important rôle in the functioning of that center, as Randall (2) has demonstrated. It was shown in this study that the use of the warm collar about the neck was effective in improving respiration under certain conditions. It is likely that the temperature of the air breathed, independent of deep body temperature, also affects the respiratory center. In these experiments, air temperatures ranged from 20 to 27.9°C. (room temperature). The effects of extreme changes of respiratory-air temperature upon the tolerance of birds to a constant degree of hypothermia, as could be produced by the water-bath method, remain to be determined.

The lethal temperature of 7-day-old chicks, according to Randall (2), is about 15°, which is approximately 7°C. below that of adult chickens. The chick embryo is poikilothermic, but at hatching time, the chick begins to develop a temperature-control mechanism, but apparently this control is not completed until the bird is about 15 or more days old, since at about that time the chick attains the body temperature of the normal adult fowl. This fact may account

for the observed differences in lethal temperatures of the young chick and the adult fowl. The lethal temperature of the nestling wren before temperature control has been developed is considerably lower than that for the adult wren, which is 21.7°C. Wiggers (6), in reviewing the effects of hypothermia in mammals, states that in most mammals the lethal temperature is about 20°C., which is about 2°C. lower than that for the adult chicken.

It was found (Sturkie, 4) that if hypothermic birds are warmed in water at 46°C. until body temperature is about normal and then dried, no ill effects result, except that laying birds usually cease laying for 3 to 7 days, depending upon the severity of the treatment. It was further demonstrated that if hypothermia is induced in hens while an egg is in the uterus, the egg is subsequently laid prematurely, but if the body temperature has been lowered and subsequently brought back to normal before the egg normally reaches the uterus, the egg is not laid prematurely. The normal time of oviposition is delayed, however. Twin-chick embryos have been produced (5) by inducing hypothermia in the hen before the egg reaches the uterus.

SUMMARY

Severe hypothermia was induced in cocks and hens by suspending the birds in water ranging in temperature from 6 to 11.7°C. The lethal body-temperatures of eight hens treated were 23.3, 23.3, 23.9, 22.8, 23.3, 23.6, 22.8 and 23.6°C., and their periods of survival were 50, 50, 65, 83, 65, 90, 90 and 79 minutes, respectively. There was little difference in the lethal temperatures of the hens, but the periods of survival were, in most hens, directly proportional to the temperature of the water.

The lethal temperatures of the four males, 19.4, 22.2, 21.1 and 20.0°C., were in every case lower than those for the hens. The periods of survival of the males were 65, 75, 77 and 90 minutes, respectively.

Moderate hypothermia was induced in five hens by placing them in water at temperatures ranging from 20 to 27.8°C. Body temperature ranged from 27.8°C. to 31.1°C. most of the time.

The lethal body-temperatures for the hens thus treated were 27.8, 29.4, 27.8, 28.9 and 28.9°C., and their survival periods were 44, 42, 40.25, 36.5 and 35 hours, respectively.

REFERENCES

- (1) BALDWIN, S. P. AND S. C. KENDEIGH. Scientific Publications of the Cleveland Museum of Natural History, vol. 3, 1932.
- (2) RANDALL, W. C. This Journal 139: 56, 1943.
- (3) RANDALL, W. C. Proc. Soc. Exper. Biol. and Med. 52: 240, 1943.
- (4) STURKIE, P. D. Poultry Science 25: 369, 1946
- (5) STURKIE, P. D. J. Exper. Zool. 101: 51, 1946.
- (6) WIGGERS, C. J. Physiology in health and disease. 4th ed., p. 940. Lea and Febiger, Philadelphia, 1944.

THE RELATION OF RENAL BLOOD FLOW TO EFFECTIVE ARTERIAL PRESSURE IN THE INTACT KIDNEY OF THE DOG

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Interest attached to renal blood flow during traumatic and hemorrhagic shock, and the significance of reduction in renal blood flow in the etiology of renal hypertension has made it desirable to know what effect changes in aortic pressure, vasomotor activity, and changes in effective viscosity of the blood have in causing observed alterations in renal vascular resistance and blood flow. Although apparently successful studies of this type have been made on the isolated hind limb of the dog (1, 2, 3), it was thought that the kidney's susceptibility to adverse conditions encountered during *in vitro* perfusion with blood and other fluids would make it desirable to pursue such studies in the intact animal. Thus, edema formation, settling or agglutination of erythrocytes, clotting, or the formation of toxic or constrictor substances which might occur in shed blood would be minimized or entirely eliminated.

The present study was accomplished with minimal disturbance to the kidney by a special method of renal vein cannulation. The relationship of effective arterial pressure to total renal blood flow was then evaluated through variation of the aortic pressure by compression of the dorsal aorta just above the renal artery. In some animals the kidneys under observation were denervated.

METHOD. Dogs anesthetized with 30 mgm. per kgm. of sodium pentobarbital administered intravenously were used in this study. Surgical procedures involved a retroperitoneal approach to the dorsal aorta and the left kidney, cannulation of a femoral artery and vein, and cannulation of both external jugular veins. The method of measuring renal venous outflow has been described in previous reports (4, 5). It consists in passing a jugular sound, perfused with saline, into the left renal vein via the vena cava. After heparinization of the animal with 4 mgm. per kgm. initial dose (5 mgm. total dose was given every half-hour thereafter), the cannula is securely ligated, and blood is permitted to pass through the cannula, and by a connecting tube is immediately passed into the opposite jugular vein. From this circuit, 10–20 cc. aliquots of blood are periodically shunted into graduated cylinders. By timing accurately the period of outflow, total renal blood flow (RBF) in cubic centimeters per minute can be calculated. In this report all flow values are expressed in terms of cubic centimeters per minute per gram of kidney mass (wet weight), facilitating the grouping of data in the figures and tables.

Because the renal cannula circuit itself offered some resistance to blood flow,

¹ Aided by a grant from the Commonwealth Fund.

this circuit had to be calibrated by *in vitro* perfusion at varying rates of flow with blood of different hematocrits warmed to 37–38°C. An approximate average value for the pressure drop across total renal circuit, from renal vein to jugular vein, including the renal cannula, would be 7.0 mm. Hg for blood of an average hematocrit of 42 per cent, at a flow of 100 cc. per minute.

The *effective mean arterial pressure* which drives blood through the kidney is defined as the arterio-venous pressure difference across the renal vessels, minus the pressure drop through the renal cannula circuit. Subsequently in the text this will be referred to as simply the "effective pressure." It is assumed that the pressure registered in the femoral arterial manometer whose "0" level is set at the level of the renal artery approximates the renal arterial pressure. The out-flow orifice for measuring renal venous flow is also set at the level of the renal artery. It should be kept in mind, therefore, that in these experiments the effective pressure is always lower than the mean arterial blood pressure, the difference being the correction necessitated by the resistance of the renal venous circuit. At control blood flows this difference averaged 17 mm. Hg, becoming proportionally less with reduction in flow.

Experimental variation in effective pressure was accomplished by compression of the dorsal aorta about 1 cm. above the left renal artery. This was done by passing a loop of cord under the aorta, then passing it to the surface of the incision through a brass sleeve. Tourniquet action resulting from twisting the ends of the cord permitted a gradual, uniform constriction of the vessel which could be rapidly released at any time. Since renal vascular reactions might have resulted from a single, progressive aortic constriction, compression was released immediately after each period of experimental observation.

Studies of the relation of RBF to effective pressure are presented from a total of thirteen animals. In five of these the kidneys were denervated. Control periods of observation were long enough to insure relative stability of mean arterial blood pressure and RBF. These periods averaged 17 minutes in length. Individual RBF determinations made every minute or two during these periods seldom varied more than 0.3 cc. per minute per gram of kidney from the mean of any control observation period. Determinations made during lower pressure periods varied even less since the periods of observation were much briefer.

Following the control observations, progressively lower levels of effective pressure were experimentally obtained by increasing the degree of aortic compression in successive stages. After reduction of pressure to any desired level, RBF determinations were made at $\frac{1}{2}$, 1, and $1\frac{1}{2}$ minutes (in some only at $\frac{1}{2}$ and 1 min.). Aortic compression was then immediately released, and effective pressure and blood flow were permitted to return to control levels, at which time flow and pressure readings were repeated usually 1 and 2 minutes after release. Experimental and control periods were then alternated until a maximal range of pressure-flow determinations was obtained. The total time for a complete series, not including the initial control period, averaged 38 minutes (28 to 50 min.) Since RBF was essentially constant during any period of observation, it appeared

that stabilization of the pressure-flow relationship had occurred within $\frac{1}{2}$ minute after alteration of pressure. This was true except at the lowest pressure levels in some experiments where a gradual continuous decrease in flow was observed throughout the period.

Green has pointed out the importance of controlling the collateral blood supply in studies of this type (3). It has been assumed that the collateral supply to the kidney is a negligible factor in this study, based on the high extraction ratios obtained for certain substances excreted by the kidney (6, 7), and by the fact that when the renal artery is occluded the renal venous outflow, as measured by the present method, is insignificant.

Hematocrit determinations of renal venous blood were made during the control period, midway, and at the conclusion of a series of observations. In only two experiments did the hematocrit volume change by more than 3 vol.-per cent during the course of the experiment: In one the change was an 8 vol.-per cent

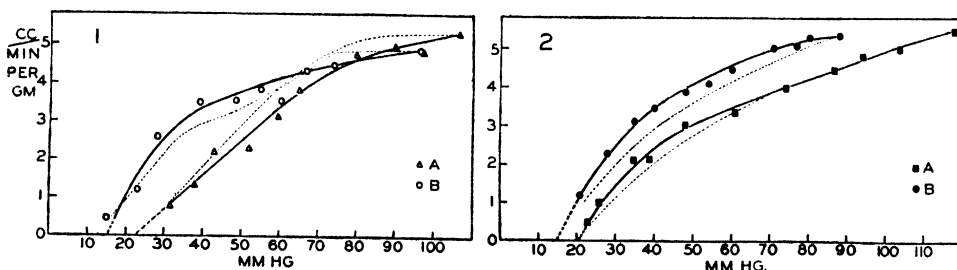


Fig. 1. The relation of renal blood flow to effective arterial pressure in kidneys with nerves intact. The dashed line shows the trend of observed data before correction for extraneous nervous and humoral influences (see text).

Fig. 2. The relation of renal blood flow to effective arterial pressure in denervated kidneys.

increase, and in another a 10 vol.-per cent increase. It is probable that changes in hematocrit of this magnitude may significantly increase blood viscosity, for the data of Whittaker and Winton (1) show that in the perfusion of the hind limb an increase in hematocrit from a value of 40 per cent to a value of 50 per cent increases the relative viscosity of blood from approximately 1.88 to 2.23, a 19 per cent increase. This effect could be corrected however, (see below) on the basis of observed deviation in subsequent control flow values from the original control average.

RESULTS. A. Effective arterial pressure-blood flow relationship in innervated kidneys. The curves relating pressure to flow in kidneys with nerves intact typically exhibited a prominent concavity toward the pressure axis (fig. 1, dashed curves). The shape of the curves shows that RBF does not significantly decrease in spite of alteration in pressure from 107 mm. Hg to 84 mm. Hg in experiment A, and 96.5 mm. Hg to 74 mm. Hg in experiment B. Since flow is maintained despite decrease in pressure, one is led to infer that a contributory

factor is an increase in the caliber of the renal vessels under these circumstances. Referring to typical experiments in table 1, it is of interest to note that this effect is maintained when aortic compression is briefly released during the subsequent control periods which intervene with the experimental periods of reduced pressure. Because of this, RBF during subsequent control periods is at first actually greater than the original control value.

TABLE 1

The relationship of effective arterial pressure to blood flow in representative experiments in kidneys with nerves intact

CONTROL				EXPERIMENTAL			
	Pressure mm. Hg	R.B.F. cc./min. per gm.	PRU*	Pressure mm. Hg	R.B.F. cc./ min. per gm. (observed)	R.B.F. cc./ min. per gm. (corrected)†	PRU
Expt. A 13 kgm. 42 gm. kidney 43.5% hemat.	107	5.31	20.2	97	5.25	4.85	20.0
				90	5.33	4.94	18.2
				80	5.16	4.78	16.7
	104	6.17	17.0	65	4.47	3.80	17.1
	94.5	6.04	15.7	59.5	3.76	3.12	19.1
	101.5	6.40	15.9	52	2.77	2.26	23.0
	102	6.45	15.8	43	2.50	2.19	19.6
	99	5.49	18.0	38	1.29	1.27	30.0
	89	4.82	18.4	31.5	0.76	0.77	41.0
	93	5.10	18.2				
Expt. B 14 kgm. 48 gm. kidney 58.2% hemat.	96.5	4.87	19.8	74	4.80	4.50	16.4
	94	5.50	17.1	60.5	3.87	3.55	17.0
	93	5.10	18.2	67	4.31	4.36	15.4
	97	4.50	21.5	55	3.59	3.83	14.4
	93.5	4.60	20.3	48.5	3.15	3.56	13.6
	94	3.98	23.6	39	2.91	3.50	11.2
	88	3.95	22.3	28	2.14	2.59	10.8
	86	3.96	21.8	23	0.98	1.20	19.2
	86	3.80	22.6	15	0.37	0.46	32.6
	90	3.80	23.7				

Initial control periods represent the average of 9 to 13 observations, while all other periods include the average of 2 or 3 observations. Renal blood flow (RBF) is expressed in terms of unit kidney mass.

* PRU (peripheral resistance units) is taken as the ratio of effective pressure/R.B.F., cc. per min. per gram.

† See text for method obtaining corrected flow.

In a study of this type it is desirable to minimize extraneous influences on blood flow which may have been actuated by the special conditions of the experimental technique. Therefore, it was thought necessary to correct the observed experimental flows for this effect, in order to preserve the relationship of the experimental periods to the original control value. This was done by taking the average of the control periods just before and after the experimental period of reduced

pressure as a ratio to the original control value for flow,² then correcting the observed flow by this ratio as follows:

$$\frac{F_c}{\frac{F_{c_1} + F_{c_2}}{2}} \times F_E = \text{corrected flow}$$

where F_c is the original control flow, F_{c_1} and F_{c_2} are the subsequent control flows taken just before and after the observed experimental flow, F_E . Data giving both observed and corrected flow are presented in the tables, and in figure 1 the solid curves represent corrected values, where they may be directly compared to the dashed curves, representing the observed data. The resemblance of the corrected curves to the curves exhibited by the denervated kidneys (compare with fig. 2 and fig. 4) suggests that the observed dilatation is of extrinsic nervous origin, and that the influence can largely be corrected by the application of the above equation. Since the manifest correction as a rule has been small, one may surmise that the extrinsic nervous influence has not been of great consequence in the present experiments.

Reference to table 1 will disclose that following the increase in control blood flow which persisted during the earlier phases of any series of pressure-flow determinations, there was usually a subsequent decrease in control flow, more marked terminally after perfusion pressure had been decreased to the lowest levels. Thus, terminal control flows were usually somewhat lower than the original control values, and were accompanied by increased renal vascular resistance in eight of the experiments, occurring in denervated as well as in non-denervated kidneys. It appeared likely that the stagnation of blood flow during the periods of fairly complete aortic occlusion had created a condition which favored reduction in subsequent control renal blood flow determinations. One may speculate that locally acting humoral vasoconstrictor substances were created by the brief intervals of renal ischemia. Increase in absolute blood viscosity, as indicated by increased hematocrit in two experiments, could be expected to create a similar trend.

Again, in order to preserve the relationship of the experimental blood flow determinations to the original control flow, this trend was corrected by application of the above equation in all experiments. The effect of this correction is apparent by comparing the solid curves (corrected) with the dashed curves (observed data) at the lower perfusion pressures in experiment B, figure 1, and in the experiments of figure 2. Experiment A, figure 1, was exceptional in that renal vascular resistance did not rise terminally, and in experiment B, figure 2, increased resistance occurred early in the experiment.

Attention may now be directed to figure 3, in which the curve describes an arithmetic mean of eight experiments performed on innervated kidneys, with

² If MABP decreased during the course of the experiment the flow value at the same pressure in the early part of the curve was substituted for the original control average, thus correcting for the effect of reduced effective pressure on the control flow.

lines at ± 2 S.D., serving to indicate the limits of variability. The typical trend relating renal blood flow to effective pressure is an exponential curve, concave toward the pressure axis, which (by extrapolation) intercepts the pressure axis at 13 mm. Hg. Interpreting the apparent relationship of blood flow to pressure, we may say that as effective pressure is decreased from an average of 116 mm. Hg, RBF is decreased at first only by small decrements. With further reduction in pressure the gradient of the curve becomes steeper with larger decrements in flow. At the lowest pressures flow virtually ceases. It is apparent that resistance to flow is great at the lowest pressures, is less at the intermediate pressures, and increases again at the highest pressures.

B. Effective arterial pressure—blood flow relationship in denervated kidneys. The curves relating flow to pressure in these experiments (fig. 2 and fig. 4), although also typically concave to the pressure axis, show an immediate decrease in flow as pressure is decreased, and elevation of the rate of subsequent control flow over the original control average does not occur (table 2). This appears to

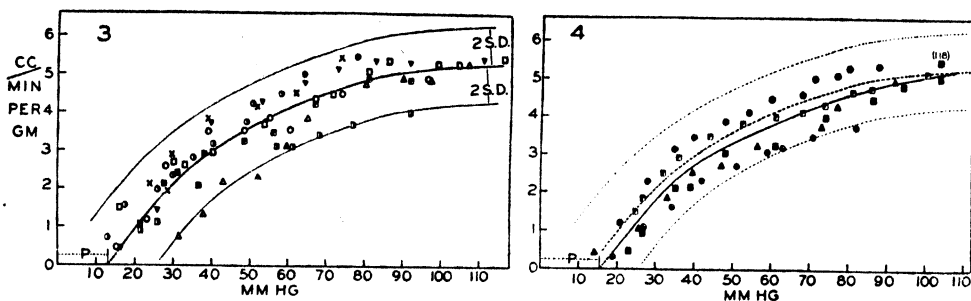


Fig. 3. The mean curve of eight experiments in kidneys with nerves intact, with lines at ± 2 S.D. (standard deviation from the mean); *P* is the indicated yield pressure.

Fig. 4. The mean curve of five experiments in denervated kidneys, shown in relation to the mean and 2 S.D. of the data of figure 3.

confirm the conclusion that the slight increase in subsequent control flow noted in the innervated kidneys is due to dilatation of reflex origin.

Individual periods of observation of five experiments are plotted with a curve representing their arithmetic mean in figure 4 in relation to the data of figure 3. It is seen that these data fall within ± 2 S.D. of the mean of the latter experiments, and that the mean curves of the two sets of data agree well, indicating that insignificant differences obtain between the pressure-flow relationship of denervated and innervated kidneys, particularly when an attempt is made to correct for extraneous nervous influence in the latter.

Interpretation of the relationship of flow to pressure in denervated preparations also shows that as the pressure is decreased from an average of 107 mm. Hg, RBF decreases at first only by small decrements. With further reduction in pressure the gradient of the curve becomes steeper and flow virtually ceases at 16 mm. Hg. Again, resistance to flow is great at the lowest pressures, is less at intermediate pressures, and increases again at the highest pressures.

C. The relationship of renal vascular resistance to effective arterial pressure. Peripheral resistance represents the ratio of the arterial pressure (or more exactly, the arterio-venous difference in pressure) to the rate of flow through the organ being perfused. In agreement with Green (3), the unit of peripheral resistance (PRU) has been defined as: $\text{PRU} = \frac{1 \text{ mm. Hg}}{1 \text{ cc./min.}}$. For convenience, renal vascular resistance has been calculated in units of cubic centimeters per minute per

TABLE 2

The relationship of effective arterial pressure to blood flow in representative experiments in which the kidneys were denervated

	CONTROL			EXPERIMENTAL			
	Pressure mm. Hg	R.B.F. cc./min. per gm.	PRU	Pressure mm. Hg	R.B.F. cc./ min. per gm. (observed)	R.B.F. cc./ min. per gm. (corrected)	PRU
Expt. A 11.5 kgm. 50 gm. kidney 44% hemat.	118	5.50	21.5	103.5	5.02	5.02	20.6
				94	4.83	4.83	19.5
	107	5.28	20.3	86.5	4.47	4.47	19.4
	107.5	5.35	20.0	74	4.00	4.00	18.5
	107	5.07	21.0	61	3.24	3.38	18.0
	97	4.60	21.0	48	2.72	3.04	15.8
	101	4.25	23.8	39	1.74	2.15	18.1
	102	3.83	26.6	35	1.65	2.13	16.4
	91	3.70	24.6	26	0.79	1.00	26.0
	85	3.63	23.4	23	0.37	0.46	50.0
	90.5	3.75	24.2				
Expt. B 15 kgm. 48.5 gm. kidney 41% hemat.	87.5	5.38	16.3	80	5.15	5.30	15.1
				77	4.94	5.10	15.1
	89	5.05	17.6	71	4.73	5.02	14.1
	88.5	5.08	17.4	68	4.32	4.60	14.8
	92	4.98	18.4	60	4.05	4.47	13.4
	89	4.76	18.7	54	3.56	4.12	13.1
	87.5	4.55	19.2	48	3.26	3.86	12.4
	94.5	4.53	20.9	40	2.82	3.46	11.6
	100	4.23	23.6	35	2.48	3.16	11.1
	96	4.19	23.0	28	1.73	2.30	12.2
	98	3.90	25.0	21	0.89	1.20	17.5
	93	4.09	22.8				

gram of kidney. Because the data of figures 3 and 4 are in good agreement, they have been combined in figure 5 and the resulting curve relating resistance to effective pressure represents the arithmetic mean of all thirteen experiments, with lines at ± 2 S.D. Resistance averages 22 PRU at 116 mm. Hg, decreasing gradually to 14 PRU at 34 mm. Hg, then increasing to 38 PRU at 16 mm. Hg. (The dashed line in the figure represents the relationship of resistance to pressure when corrected for indicated yield pressure, and will be considered more fully below.)

DISCUSSION. A. *Factors which influence the flow of blood through the kidney.*

For the laminar flow of homogeneous liquids through small tubes, Poiseuille's law states (8):

$$Q = P \cdot \frac{\pi R^4}{8L} \cdot \frac{1}{\eta}$$

where in general terms Q is the volumetric flow, P is the pressure difference between the two ends of the tube, R is the radius of the tube, L is the length of the tube, and η is the absolute viscosity of the liquid. Resistance is then defined as the pressure per unit rate of flow:

$$\text{Resistance} = \frac{P}{Q} = \frac{8L}{\pi R^4} \cdot \eta$$

It is seen that resistance is the product of two terms: the characteristics of the tube, and the viscosity of the fluid.

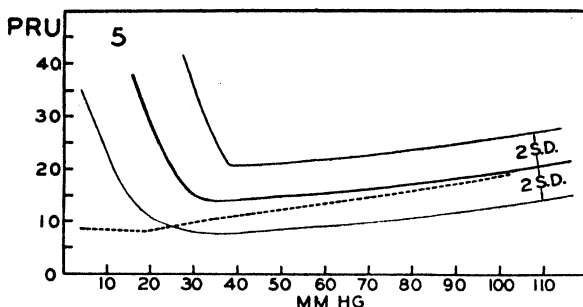


Fig. 5. The relation of renal vascular resistance (PRU: peripheral resistance units) to effective arterial pressure taken as the mean of all experiments (fig. 3 and fig. 4). The dashed line represents the renal resistance corrected for the average indicated yield pressure, 14 mm. Hg.

The significance of changes in dimensions of the tube (i.e., caliber of the vessels) can be appreciated by considering data derived from perfusion of the hind limb of the dog (1, 2, 3). The data of Green (3) show that when the hind limb is perfused under conditions which he feels represent constant states of vascular tonus, a parabolic relationship exists between pressure and flow, and in his experiments the curve is typically convex rather than concave toward the pressure axis, so that flow through the limb continuously increases per increment of pressure throughout the range observed. Although the proportionally greater flow at higher pressures may be in part due to a decrease in the effective viscosity of the blood, Green feels that more significant is the increase in radii of the blood vessels with rising internal pressure, thus reducing the resistance to flow. An alternative explanation is offered, that with increasing perfusion pressure progressively more capillaries are irrigated. Green has criticized the apparently linear curves (at higher pressures) of Whittaker and Winton (1), and Pappenheimer and Maes (2) on the grounds of abnormal dilatation of the vessels and (or) too limited a range of perfusion pressures.

In attempting to apply Poiseuille's law to flow through the kidney, one must

then first inquire as to whether or not the caliber of the renal vascular bed remains constant with varying internal pressures. The possibility of changes in the caliber of the renal vessels has been tested on isolated kidneys perfused with a homogeneous fluid, 1.5 per cent pectin solution,³ at various pressures (viscosity should be independent of flow for homogeneous fluids). It was thought that the use of pectin solution would minimize edema formation. When pectin solution is used for perfusion the curve relating pressure to flow is, in fact, parabolic in shape and convex to the pressure axis, signifying that the caliber of the renal

TABLE 3

The relationship of perfusion pressure to the flow of pectin solution through the isolated kidney

PERFUSION PRESSURE MM. HG	CC./MIN. PER GM.	PRU PER GM.	$\frac{R_1^*}{R_2}$
120	2.45	49.0	1.000
110	2.20	50.0	1.005
100	1.95	51.3	1.010
90	1.72	52.5	1.016
80	1.47	54.4	1.026
70	1.23	57.0	1.040
60	1.00	60.0	1.052
50	0.80	62.5	1.063
40	0.58	69.0	1.088
30	0.39	77.0	1.120
20	0.23	87.0	1.153
10	0.10	100.0	1.200

These data are the averages of a dozen experiments in which a series of pressure-flow determinations were done by beginning perfusion at levels above 120 mm. Hg, then as rapidly as possible decreasing the pressure to 0. A series of about 10 observations were made in each experiment during time intervals of about five to fifteen minutes.

* This is the ratio of the effective radius (R_1) of the renal vessels at 120 mm. Hg to the radius (R_2) at the successively lower perfusion pressures. (See text.)

vessels is indeed greater at the higher perfusion pressures. An attempt was made to quantitate the degree of change by application of the following formula:

$$\frac{R_1}{R_2} = \sqrt[4]{\frac{I_1 \cdot P_2}{I_2 \cdot P_1}} \quad (\text{for derivation, see Lamport (9)})$$

where R_1 , I_1 and P_1 are taken respectively as the effective radius, flow, and pressure at a maximal perfusion pressure of 120 mm. Hg, and R_2 , I_2 and P_2 are taken respectively as the radii and flows at successively lower perfusion pressures. The average of about a dozen curves shows that the effective radius (total caliber) of the vessels at 120 mm. Hg is 1.2 times as great as the total effective radius at 10 mm. Hg and, as a consequence, the resistance to flow is approximately twice as great at 10 mm. Hg as it is at 120 mm. Hg (see table 3).

³ The pectin solution (average M.W., 49,000) was kindly supplied by the Cutter Laboratories of Berkeley, California. When used, the pH was adjusted to 7.3-7.4, and perfused at a temperature of 37-40°C. Its relative viscosity was approximately 3.0X distilled water, as determined with an Ostwald viscosity pipette at 25°C.

These findings in isolated kidneys should be interpreted with caution, however, for the rate of flow of pectin solution, although less viscous than blood, averaged somewhat less than half the rate of blood flow through the intact kidney, viz., 2.45 cc. per minute per gram for pectin at 120 mm. Hg. It is believed, however, that the evidence favors the probability that the caliber of the renal vessels increases with increased internal pressure. In the absence of change in apparent viscosity of the perfusion fluid, this should result in proportionally larger flows at higher perfusion pressures, as is the case in the hind limb. It is evident from inspection of the curves that this is not the case in the intact kidney naturally supplied with blood. For an explanation of the concave nature of these curves one must examine the characteristics of the apparent (effective) viscosity of blood as it is related to rate of flow through the kidney.

Examination of the kidney pressure-flow curves in figures 3 and 4 shows that for blood of hematocrit averaging 42 per cent the extrapolated curves cut the pressure axis at an average value of 14 mm. Hg. This signifies that part of the applied pressure is used in producing viscous flow, and this has been termed the "yield pressure." (We are unprepared to say definitely that this results from "plastic flow," as the term applies to suspensoids, or that it results from interaction of the red cells to the walls of the capillaries. See Lampport (9) and Green (10) for discussion.) Lampport (11) has applied a correction for yield pressure in his calculation of renal afferent and efferent arteriolar resistances, and has arbitrarily taken a value of 20 mm. Hg, derived from Whittaker and Winton's data on the hind limb (1). If a correction for indicated yield pressure (14 mm. Hg) is applied in the data of figure 5 the resulting curve (dashed line), although almost linear, still shows a gradual increase from 8 PRU at 20 mm. Hg to 19 PRU at 102 mm. Hg. It is evident that some additional factor (or factors) operates which causes the pressure-flow relationship of the renal vessels to deviate from Poiseuille's law.

Winton (12) and Lampport (13) have made the significant observation that increased glomerular filtration at higher arterial pressures increases the viscosity of blood in the post-glomerular vessels more than at low pressures. Winton's curve relating pressure to flow in the heart-lung-kidney preparation bears a close resemblance to experiment A, figure 2 (14). Lampport has employed the clearance of inulin to aid in a quantitative assessment of the degree of hemoconcentration and increased viscosity of the blood. The implication is that blood of significantly greater viscosity would pass through the distal part of the glomerular capillaries, through the efferent arterioles, and through the proximal part of the peritubular capillaries. Reabsorption of 99 per cent of the filtered fluid in the peritubular capillary bed would of course restore the viscosity to normal on leaving the renal vein.

The increase in viscosity of blood flowing through the kidney at the higher arterial pressures is in agreement with the present experimental observations, for it has been shown that resistance to RBF is greater as pressure is increased (fig. 5). The explanation that this increased resistance is due to increased viscosity of blood resulting from glomerular filtration can be tentatively accepted.

Accepting this assumption, the curve of figure 5 would suggest that glomerular filtration begins at 34 mm. Hg (solid curve, uncorrected for yield pressure) and gradually increases at a relatively constant rate throughout the range of pressures observed. It should be kept in mind, however, that the curve describing renal vascular resistance, excluding possible effects of plastic flow, is in all probability the summation of two opposite influences, namely, the decrease in resistance resulting as vessels dilate with rising internal pressure, and the simultaneous increased viscosity of the blood. Since the net resistance continually rises at the higher pressures, the latter effect would appear to predominate.

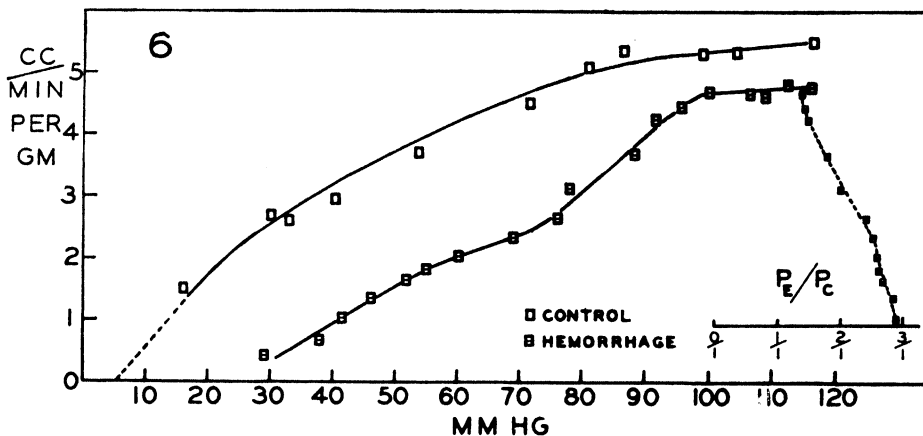


Fig. 6. A representative experiment showing the effect of hemorrhage (2 cc./kgm./2 min.) on renal vasomotor activity. Increased vasomotor activity is indicated by increase in the ratio P_E/P_C , where P_E is the experimental pressure required to produce the same flow as at control pressure P_C .

B. The estimation of renal vasomotor activity. It is evident that renal resistance can change when arterial pressure is altered in the absence of vasomotor activity due to nervous or humoral agents. This is evidenced by the higher resistance at low pressures, perhaps resulting from the plastic flow of blood, and the elevated resistance at high pressures, which is partly at least a consequence of increased viscosity of the blood resulting from glomerular filtration. Keeping in mind the curve relating resistance to effective pressure in figure 5, the decrease in resistance as pressure is reduced from the highest levels is not necessarily the result of vascular dilatation, nor is the increase in resistance at pressure levels below 30 mm. Hg necessarily the result of vascular constriction.

However, as Green (15) has pointed out, it is possible to make estimates of changes in vasomotor tone if one compares the relationship of experimental flow to pressure with the change which would have resulted by changing effective arterial pressure alone. By comparison with such a control curve, in which vasomotor tone has remained constant in a normal state, vasomotor activity during the experimental state may then be expressed as follows: α , by computing the ratio of flow in the control state (F_C) to flow in the experimental state (F_E)

at each pressure, F_c/F_x ($P_c = P_x$), or b , by computing the ratio of pressure in the experimental state (P_x) to that required in the control state (P_c) to produce the same rate of flow, P_x/P_c ($F_x = F_c$). Green feels that the second method, b , is more expedient because this ratio is almost constant over a wide range of flows, while the former, a , is not the same at all pressures.

In figure 6 appears a plot of the effect of hemorrhage on renal vasomotor tone where it may be compared with a control pressure-flow curve established just prior to bleeding. Increased vasomotor tone has been plotted by computing the ratio, P_x/P_c , as in b above. In the experiment of figure 6 there was a progressive increase in vasomotor tone as more blood was removed.

C. The constancy of renal blood flow. The apparent constancy of renal blood flow in the face of fluctuation in arterial blood pressure has been noted by a number of workers since the original observations of Rein (for discussion see Homer Smith (16)). The concept that some intrinsic autonomous regulatory mechanism exists was implied by the findings of Opitz and Smyth (17), and Hartman, Orskov, and Rein (18) who noted that a reasonable constancy of flow obtained even in denervated kidneys. However, reference to salient features of the pressure-flow relationship in the kidney suggests that no specific regulatory mechanism in a vasomotor sense needs be invoked. In figure 3, RBF is 5.33 cc./min./gm. at an effective pressure of 116 mm. Hg, decreasing to 4.8 cc./min. gm. at 80 mm. Hg. Thus, RBF changes only 9.5 per cent, while pressure changes 31 per cent. Similarly in figure 4, flow is 5.2 cc./min./gm. at 107 mm. Hg. decreasing to 4.6 cc./min./gram. at 80 mm. Hg. Here RBF changes 11 per cent while pressure varies 25 per cent. If the underlying premise is accepted that glomerular filtration rate varies with effective arterial pressure and that as a result of this blood viscosity varies directly with pressure, a ready explanation of the apparent autonomous regulatory mechanism of the kidney is offered, for increased viscosity of the blood should counteract the effect of rising pressure on flow, while decreased viscosity of the blood should oppose the effect of falling pressure on flow.

SUMMARY AND CONCLUSIONS

1. The typical trend relating renal blood flow to mean effective arterial pressure in the intact kidney is an exponential curve concave toward the pressure axis which (by extrapolation) it intercepts at an average value of 14 mm. Hg. This value becomes an acceptable figure for indicated yield pressure for blood flow through renal vessels.

2. In interpreting the apparent relationship of blood flow to pressure, one may say that as effective arterial pressure is decreased from an average of 116 mm. Hg, renal blood flow is decreased at first only by small decrements. With further reduction in pressure the gradient of the curve becomes steeper, with larger decrements in flow. At the lowest pressures, flow virtually ceases.

3. Analysis of renal vascular resistance shows that resistance to flow is quite large at the higher pressures, becomes less as pressure falls, and increases again at the lowest pressures.

4. The higher resistance at maximal pressures is probably the result of increased viscosity of blood resulting from glomerular filtration. At minimal pressures the increased resistance may be the result of development of plastic flow of the blood.

5. The shape of the pressure-flow curve in the kidney suggests that autonomous control of renal blood flow during reasonable changes in mean arterial blood pressure can largely be taken care of by physical factors, and that no specific regulatory mechanism in a vasomotor sense needs be invoked.

REFERENCES

- (1) WHITTAKER, S. R. F. AND F. R. WINTON. *J. Physiol.* **78**: 339, 1933.
- (2) PAPPENHEIMER, J. R. AND J. P. MAES. *This Journal* **137**: 187, 1942.
- (3) GREEN, H. D., R. N. LEWIS, N. D. NICKERSON AND A. I. HELLER. *This Journal* **141**: 518, 1944.
- (4) SELKURT, E. E. *This Journal* **145**: 376, 1946.
- (5) SELKURT, E. E. *This Journal* **145**: 699, 1946.
- (6) CORCORAN, A. C., H. W. SMITH AND I. H. PAGE. *This Journal* **134**: 333, 1941.
- (7) PHILLIPS, R. A., V. P. DOLE, P. B. HAMILTON, K. EMERSON, JR., R. M. ARCHIBALD AND D. D. VAN SLYKE. *This Journal* **145**: 314, 1946.
- (8) POISEUILLE, J.-L.-M. *Ann. de Chim. et de Physiol.* 3d ser. **7**: 50, 1843.
- (9) LAMPORT, H. *Howell's Textbook of physiology*. 15th ed., (Saunders) Ch. 30, p. 630-59, 1946.
- (10) GREEN, H. D. *Circulation: physical principles. Medical Physics (Year Book Publishers)*, p. 208, 1944.
- (11) LAMPORT, H. *J. Clin. Investigation* **22**: 461, 1943.
- (12) WINTON, F. R. *Trans. XIVth Congresso Internaz. de Fisiol.*, p. 264, 1932.
- (13) LAMPORT, H. *J. Clin. Investigation* **20**: 535, 1941.
- (14) WINTON, F. R. *Personal communication*.
- (15) GREEN, H. D., R. N. LEWIS AND N. D. NICKERSON. *Proc. Soc. Exper. Biol. and Med.* **53**: 228, 1943.
- (16) SMITH, H. W. *The Harvey Lectures, Series XXXV*, p. 166, 1939-1940.
- (17) OPITZ, E. AND D. H. SMYTH. *Pflüger's Arch.* **238**: 633, 1937.
- (18) HARTMAN, H., S. L. ORSKOV AND H. REIN. *Pflüger's Arch.* **238**: 239, 1936.

A STUDY OF NERVE DEGENERATION AND REGENERATION¹

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Though the progression of the functional changes occurring during the degeneration and the regeneration of nerve has been very much investigated, examination of recent studies reveals a number of inconsistencies that remain to be explained. For example, Titeca (1935) concludes that excitability to induction shocks is the same in normal and degenerating nerve and disappears in all parts of the degenerating nerve at the same time, whereas Rosenblueth and collaborators (1939; 1943), confirming Parker (1933), conclude that degeneration progresses centrifugally. And, according to Berry, Grundfest and Hinsey (1944), conduction rate in regenerating fibers does not ordinarily change with the distance beyond the union, yet it is known that the diameters of the regenerating fibers exhibit a peripheralwards taper (Gutmann and Sanders, 1943) which should, on the basis of the relation that is believed to obtain between fiber diameter and conduction velocity, reveal itself as a corresponding taper of the conduction rate. It seemed that if these actually are discrepancies they might be referable to inadequacy of the methods used in controlling observations. Rosenblueth *et al.* employed as the control the companion nerve of the opposite side, and Berry *et al.* the part of the nerve central to the union.

It occurred to us that the observations would be better controlled if they could be made on normal and altered fibers in one and the same nerve trunk, for then the results could be expressed as ratios of which the denominators would be the values given by the normal fibers under identical conditions.

With this in mind the first requisite was to find a long and unbranched, or practically unbranched, nerve arising from the central nervous system by two (or more) uniform, unbranched roots. With such a preparation one of the roots could be crushed and, during the degeneration or regeneration of those fibers, all of the fibers in the nerve formed by their union could be stimulated and records taken separately of the action potentials in the two roots, one made up only of the responses of the altered fibers, the other of the responses of the normal fibers. The present study is based primarily on observations thus controlled, though additional observations have been made by methods previously employed by others.

METHODS IN GENERAL. Of the nerves examined, namely, sciatic of the frog, obdurator and phrenic of the dog, the last supplied best the requisites of the method. Usually it is formed by the union of three roots derived, one each, from the Vth, VIth and VIIth cervical nerves. Ellenberger and Baum (1891) picture

¹ The work described in this paper was done in part under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Washington University.

the arrangement of the roots as indicated in figure 1a. After we had obtained some results that were inconsistent with this arrangement of the roots it was ascertained that the VIIth joins the VIth root as indicated in figure 1b. The VIIth root generally is very minute and inaccessible, and as a consequence it was not seen in the preliminary experiments until certain anomalous results revealed the arrangement shown in this figure. Thereafter in the routine procedure for the study of degeneration the Vth root was crushed, the fibers of the VI root serving as the control, and for observations on regeneration the VIth root was crushed, the fibers of the Vth root then serving as the control.

The loci of the crushes were marked by loosely tied ligatures. The anesthetic was intravenous nembutal. After suitable intervals the anesthetized animals were sacrificed by bleeding and the whole length of the phrenic was excised along with its Vth and VIth roots. The lengths available for study sometimes exceeded 200 mm. The phrenic trunk is less than 1 mm. in diameter. It is surrounded by a thick sheath which is attached to the nerve by loose areolar tissue. With care this sheath can be dissected away fairly cleanly, but not without running some risk of damaging the nerve. As a rule, therefore, the nerve was

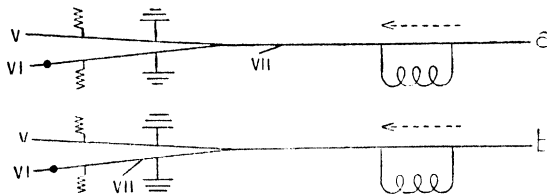


Fig. 1. Diagram to show (1) the union of roots from the cervical plexus to form the phrenic nerve, *a*, as described by Ellenberger and Baum, *b*, as we found it to be, and (2) the arrangement of electrodes.

cleared of this sheath only at places to which recording electrodes were to be applied. The roots are, of course, slenderer still. It is possible, however, to prepare them reasonably easily and fairly clear of adherent tissue, excepting when they pass through scar tissue, as is the case in the regeneration experiments.

The recording and stimulating electrodes have been of the kind previously used in this laboratory. For the comparative observations the excised preparation is mounted horizontally in a specially constructed moist chamber, on electrodes placed as indicated in figure 1. With electrodes arranged as shown in the figure, the phrenic can be stimulated with single shocks at selected loci along its length while recording monophasically from each of the roots separately. Usually the part of the preparation first to be stimulated was the distal end, then in successive steps points located more proximally, and finally, in order to check the stability of the preparation, the distal end again. In a few of the experiments the roots were stimulated separately while recording from points along the nerve trunk. The stimuli usually were repeated approximately at the rate of one a second and photographs were made of single sweeps across the face of the cathode ray oscillograph. The temperature was that of the room, usually 26 to 27°C.

The results obtained by the comparative method are expressed as ratios: $\frac{\text{response from the altered fibers}}{\text{response from normal fibers}}$, usually multiplied by 100. In this way the effects of all extraneous conditions that affect both sets of fibers alike, such as temperature, spread of stimulating current, etc., are cancelled.

DEGENERATION. Since, as will be seen, degeneration is complete in 96 hours the full length of the nerve, most of the observations have been made on preparations degenerating for about 72 hours. The curves plotted, unless otherwise specified, are based on data derived from the 72 hour nerves.

Excitability. Methods. As in previous investigations in this laboratory the stimuli were condenser discharges initiated by a gas discharge tube, activated through a relay circuit by the sweep of the cathode ray tube, and delivered to the nerve through a low-capacity, shielded transformer. The shock strength was controlled potentiometrically by a Leeds Northrup-Kohlrausch slide wire with 100 scale divisions readable to 0.1 division. For each pair of readings an external resistance was adjusted so as to bring the position of the slide into the part of the scale where the instrument had the greatest sensitivity when the stimulus had roughly half-maximal strength. When the relative excitabilities of a pair of determinations were widely different, as in the early stages of regeneration, a shunt was added in parallel after making the reading from the normal fibers, thus increasing by a known factor (approximately 10) the sensitivity of the instrument in the low part of the scale.

For the determination of excitability two end points have been used, namely, half-maximal and threshold responses, and the results are expressed as the reciprocals of the ratios $\times 10,000$.

The half-maximal method. A half-maximal response is one that has half the area elicited by a maximal stimulus. Since, however, it is not practicable to measure areas during the course of an experiment, the strength of current was first determined that elicits a spike of maximal amplitude and then the strength was determined that elicits a spike of half that height. This criterion, however, is affected by the distance of conduction: as the distance decreases fibers of low excitability (and slower conduction rate) come to contribute more and more to the height of the action potential. Due to this effect it was necessary to rather arbitrarily use as the "maximum" end point that strength of current beyond which further increase increased the spike height only slightly. Despite this difficulty, the end point for the half-maximal of these maximal spikes is quite sharp; repeated readings match within 1 to 2 per cent (see, for example, fig. 7). Our main reliance is placed on data derived through this procedure.

Results, half-maximal method. In figure 2 are plotted in the upper graph the results derived from eight normal preparations and in the lower the results from degenerating preparations, seven after 72 hours and one after 89 hours (heavy broken line). For the normal (the upper) group of curves the abscissas are the distances of conduction; for the degenerating (the lower) group they are the distances, lesion (placed at zero) to stimulator. Since the injured locus is on the root in the neck, whereas the junction of the Vth and VIth roots ("the crotch")

is located inaccessibly at variable distances down in the thorax, it was necessary in many of the experiments, in order to derive data from points closer to the lesion than the distance, lesion-to-crotch, to lay the two roots for some distance together on the stimulating electrodes, as indicated in the lower inserted diagram of the nerve. The "crotch" in these figures is placed approximately in the position it occupied in the average experiment. Excitability ratios determined with

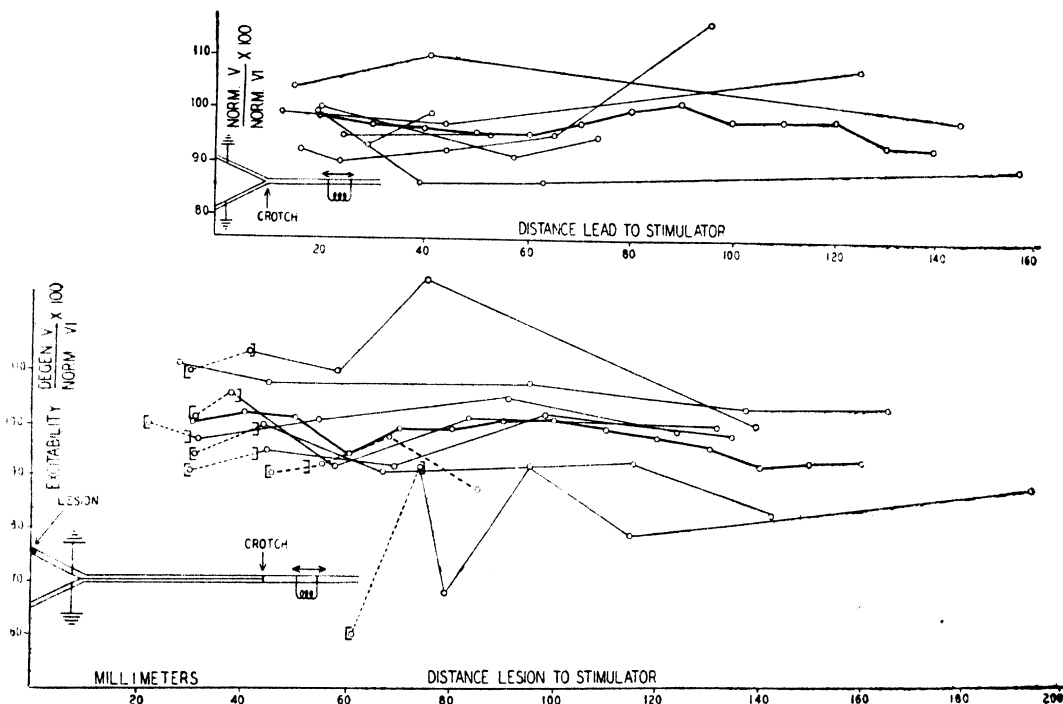


Fig. 2. Relative excitability, half-maximal method. *Upper graph*,—normal (Vth root) fibers *vs.* normal (VIth root) fibers. *Lower graph*,—continuous lines: Vth root fibers degenerating 72 hours *vs.* normal VIth root fibers. Heavy broken line curve: 89 hour preparation. The brackets indicate the stretches through which the stimulating electrodes were on the juxtaposed roots. The upper, or normal, graph is so placed over the lower, or degenerating, graph that the crotches of the two preparations are roughly on the same abscissa.

The arithmetic means of each of the two sets, excluding the 89 hour preparation, are given by the heavy lines.

The more irregular curves are from preparations that required the highest amplifications.

the stimulator on the roots thus placed in juxtaposition may not be as strictly comparable as are the data from determinations with the stimulator on the nerve trunk distal to the crotch, where the fibers are intermingled in a common sheath. The parts of the curves derived from juxtaposed roots are indicated in the graphs by the dotted portions included in brackets.

In each of the graphs the heavy curve is the mean of all of the comparable

determinations. In view of the spread of the individual curves and of their irregularities (this is especially true in degeneration) the mean has only an approximate value; and, due to the limited number of observations, it is without significance in degenerating preparations beyond the distance of 125 mm., and beyond 70 mm. in normal preparations.

The mean derived from the normal preparations (upper set) fluctuates between 98 and 93, but in the region where there is an adequate number of determinations, namely, between about 20 and 70 mm., the range is between 98 and 95 and is without any definite directional trend. The mean from the degenerating preparations has a similar range, but aside from certain irregularities, seems to have a slight downward trend particularly at the longer distances. The 89 hour curve (broken line) lies within both the normal and the 72 hour ranges, though low, its most distal point being the lowest. It would be precarious to draw any definite conclusions from this single observation. However, it does seem justifiable to conclude from the entire picture that if there is any trend during degeneration it is in the direction of a slight lowering of excitability peripheralwards and a slight lowering of excitability with increasing degeneration time. The fact that the averages from the normal nerves are in general somewhat below one hundred presumably is the expression of the normal difference in the sizes of the two roots: since VI usually is slightly larger in diameter than V, the VIth root on a statistical basis would carry some fibers that are larger than any in the Vth.

The end point by the *threshold method* of determining excitability is the smallest visible deflection with the highest available amplification. Since with our preparations the amplification could not be increased sufficiently to bring into evidence the spike of a single axon it is possible that the sensitivity of this method decreased *a*, as the distance increased (due to increasing dispersion of the spikes of the most excitable fibers) and *b*, as the total number of the fibers conducting to the lead decreased (since the smaller the number of responding fibers the smaller would be the chances that there would be outstandingly large fibers).

Results. In view of the probable inadequacies of the threshold method it will, therefore, suffice to state merely that the mean curve derived from the normal preparations fluctuates between 96.5 and 91 with a slight downward trend, and that the mean curve from the 72 hour preparations slopes downward to 82.5 from an initial value of 99.

Discussion. The finding of Rosenblueth and Dempsey that chronaxie is not altered appreciably during degeneration we can confirm. Those authors found, however, that the rheobase is higher in degenerating nerve, and, though they realize that this may be due to the "edematous condition" of the degenerating nerve, they nevertheless conclude that degenerating nerve even at 2 days is less excitable than normal nerve. Since in our preparations, the normal and the degenerating fibers are mixed in the same nerve trunk, their environment presumably is identical. Under such circumstances differences in current duration might alter the magnitude but not the sign of any difference in excitability. We, therefore, conclude that no significant change in excitability can be detected at

least in the more proximal reaches, even when, as will become apparent below, the area ratio has fallen to less than 1/100th its normal value, though in the more distal reaches of the preparations excitability may be declining (see below).

Conduction velocity. Method. For the determination of conduction rates the stimuli have been just maximal for each set of fibers and as a rule the amplifications have been adjusted so that the heights of the records to be compared were approximately alike (see, e.g., fig. 7). Conduction time, the time elapsing be-

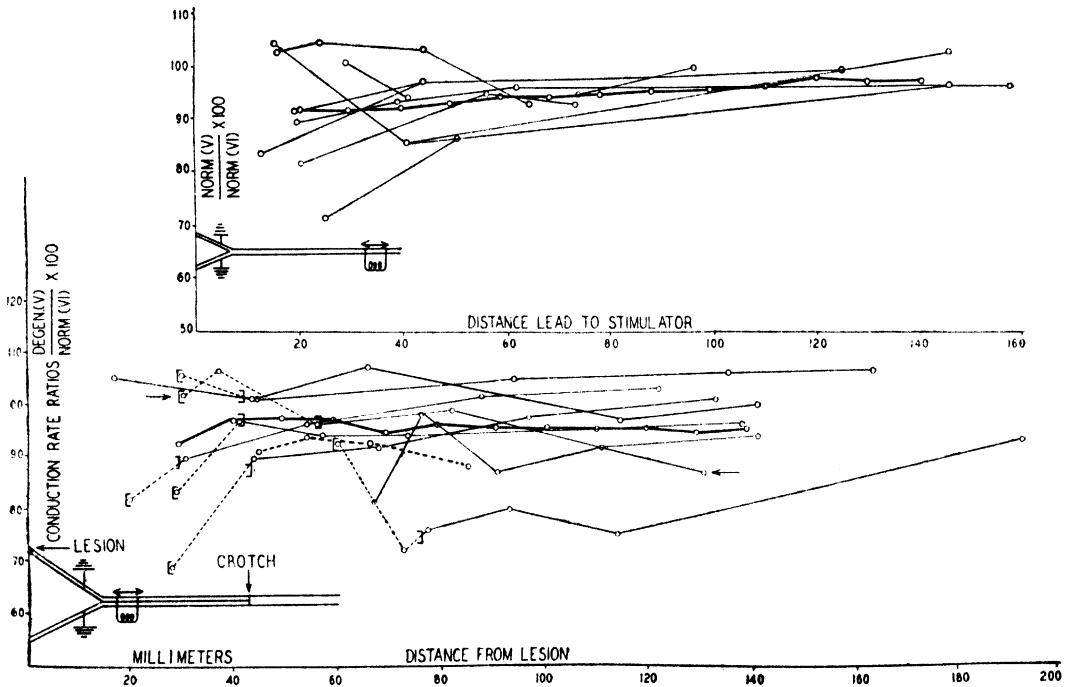


Fig. 3. Relative conduction velocities. *Upper graph*,—normal (Vth root) fibers vs. normal (VIth root) fibers. *Lower graph*,—Vth root fibers degenerating 72 hours vs. normal VIth root fibers. The brackets include the stretches through which the stimulator was on the juxtaposed roots.

The heavy line curves are the means.

Heavy broken line curve: 89 hour preparation.

tween the start of the shock artifact and that of the action potential, was measured on enlargements of the records.

The *results* have been plotted in two ways. *a.* In order to make possible the presentation of all of the data in a single figure they have been plotted in figure 3 as the ratios of the conduction times against the distances. The slopes of these curves indicate the sign, but not clearly the magnitude, of the changes in conduction rate with the distance. Horizontal curves signify that if the velocity is changing with the distance in one of the sets of fibers, it is changing in like manner in the other set. *b.* In addition all of the data from each set of fibers have been

plotted in the usual manner, as the conduction distance *vs.* conduction time. Two of these graphs are reproduced as figures 4 and 5 (see below).

Normal nerves. The data from the normal nerves, plotted in the upper set of curves of figure 3, are the conduction rate ratios against the distances of conduction. Beyond a distance of about 50 mm. from the lead the curves all run fairly close together, the spread between them amounting to little more than 10 per cent. At shorter distances of conduction the spread is greater, due presumably to increasing error of measurement resulting from the encroachment of the shock artifact and from diminishing conduction times. The mean of all the normal determinations, the heavy curve, is approximately a straight line possibly sloping upwards from 92 to 98. This upward slope, if significant, means that the conduction velocity is relatively somewhat faster in the more peripheral parts of the fibers supplied by the Vth root. What this may signify is not clear. The average ratios all are less than 100, presumably because the VIth root, being on the average somewhat larger than the Vth, will on a statistical basis contain larger fibers than the Vth.

The data from the degenerating preparations, plotted in the lower set of curves of figure 3, are the conduction rate ratios against the distances of the stimulator from the lesion. These curves are in general less regular than those from the normal nerves and are less closely bunched. This is due mainly to the fact, as will be seen, that the spike potentials from the degenerating roots are much lower than those from the normal fibers, so that higher amplifications are required for the former, and the records therefore may lack the smoothness of those of the normal spikes. Thus with normal preparations the amplifications were usually as 1:1 and never exceeded 2.6:1, whereas with the degenerating preparations the amplification ratios for the 72 hour nerves ranged between 3.3:1 and 10:1; and for the 89 hour nerve it was 44:1. Despite the irregularities of the individual curves the mean curve, if the first point be disregarded, is reasonably smooth and runs approximately from 96 down to 94. The first point on the curve is below this level, but for this determination the stimulating electrode in every case save one was on the approximated roots, where stimulus spread is not perfectly controlled. When the individual curves from 72 hour preparations are examined it is seen that there is only one that slopes downward to any considerable degree, namely, the one marked by arrows. The slight downward slope of the curve of averages is due almost entirely to the data from this experiment.

Figure 4 is an example of the velocity curves. It is based on the 72 hour experiment, just referred to, whose curve definitely deviates from the horizontal. The rates here, normal and degenerating, are alike at the shorter distances, 25.3 mps., but at the longest distance the rate in the altered fibers falls to 17.3 mps. Figure 5 shows the conduction rates in the 89 hour preparation. The rate in the normal fibers is 35 mps. throughout. In the degenerating fibers the rate through the first 15 mm. is approximately the same as in the normal fibers, but falls off to 33 mps. at 34 mm. and to 24 mps. at 51 mm.

Discussion. The two curves of the averages of conduction velocities against distance then are at the same general level, but the normal curve possibly slopes

upwards slightly, while the curve from the 3 day nerves possibly slopes downwards. The conduction curve from the 89 hour nerve lies within the range of

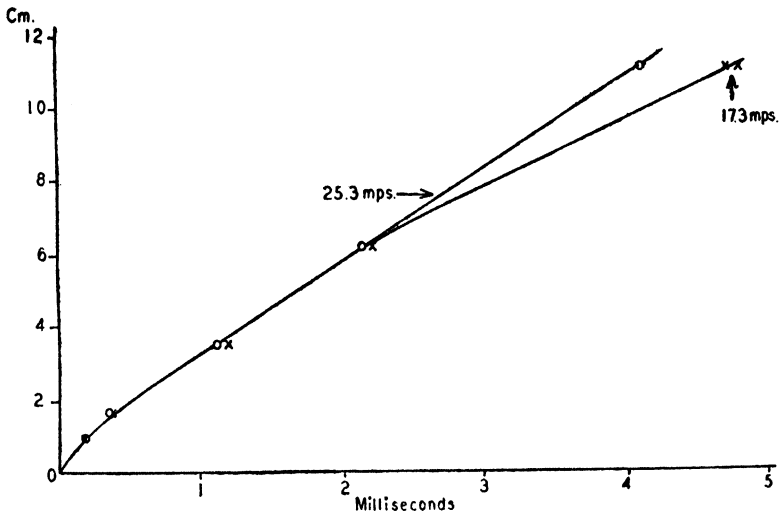


Fig. 4. Conduction velocities in the normal (circles) and in the degenerating (crosses) fibers of the only 72 hour curve of figure 3 (marked by arrows) that has a definite downward slope.

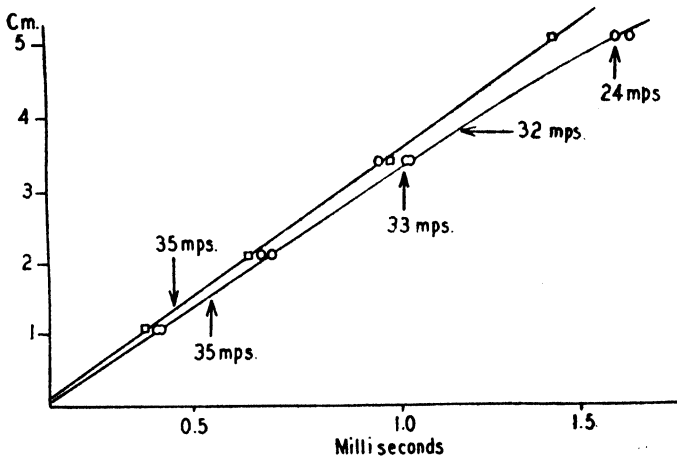


Fig. 5. Conduction velocities in normal (circles) and in degenerating (crosses) fibers of the preparation degenerating 89 hours, the heavy broken line curve of figure 3. For distance from lesion add 34 mm.

those from the 72 hour preparation but rather low. It definitely slopes downwards. Though the decline of the mean curve from the degenerating preparations is vanishingly slight, it may be significant when compared with the slight

upward slope of the normal curve and possibly signifies that there is some decline of the measured conduction rate toward the periphery in degenerating nerves. The comparative method fails to disclose a peripheralwards increase in the rate of conduction, such as Rosenblueth and Dempsey (1939) describe.

Action potential areas. Method. Two procedures have been used in securing area data. *a.* For the most part, as usual, two leads have been fixed, one on each of the roots, and the stimulator has been shifted along the nerve. The disadvantage attaching to this procedure is that one cannot be certain that the two leads will remain equally monophasic throughout a set of observations. However, that they probably do is indicated by the fact that the curves from the normal preparations all run essentially horizontally. *b.* In order to check this factor, in a few experiments the procedure has been to stimulate the roots separately while leading from the nerve trunk. In successive observations the leads are moved closer to the stimulator, each time killing the trunk between the lead as to render the records monophasic. This procedure has the disadvantage that it cannot be repeated. The area graph (fig. 6) includes two observations of this kind, namely, the curve marked 69 (from a 69 hr. preparation) and the two curves indicated by arrows, both from the same preparation. Of the latter, the method for the upper record was the usual one, for the lower record, the method described above. Excepting a difference in levels, these two curves are essentially alike.

In order to secure records that would suffice for the accurate derivation of areas, and of areas that would be comparable, the amplifications and the sweep speeds in each experiment were adjusted so that the action potentials were ample and as broad as possible and located as close to the center of the tube film as was consistent with keeping the shock artifact on the screen. The areas of the enlarged records were measured with a planimeter and any adjustments of sweep speed and/or amplification that were made during a set of observations were cared for by multiplying the measured areas by their respective amplification factors and dividing the result by the respective lengths of a unit of time, say, 1 msec., laid off approximately under the middle of the spikes. Stimuli were just maximal for both sets of fibers. Since conduction rates are not altered materially by degeneration lasting 72 hours it may be assumed that individual axon spikes likewise are not, and therefore that the areas are determined almost exclusively by the number (and the sizes) of the fibers conducting from the stimulated locus to the lead.

The results from the normal preparations are indicated by circles in figure 6. The wide range of the levels at which these curves lie is the expression of at least three factors, namely, anatomical differences in the sizes of the two roots, possible damage of one or the other of the roots done in preparation and differences in the amount of adherent connective tissue under the two leads. The essential observation is that with but one exception, for which there is no explanation, the curves are relatively smooth and horizontal. Despite the great differences in levels, the mean of all the ratios gives a curve that lies in the proximity of 100. This, however, is of little significance in view of the small number of observations available. The fact that the curves run horizontally is significant because it

proves that such branching as there may be of the phrenic in the thorax does not alter appreciably the areas of the spikes derived from the Vth and the VIth roots. It signifies also that if the leads become less monophasic with the passage of time, the change affects both leads alike.

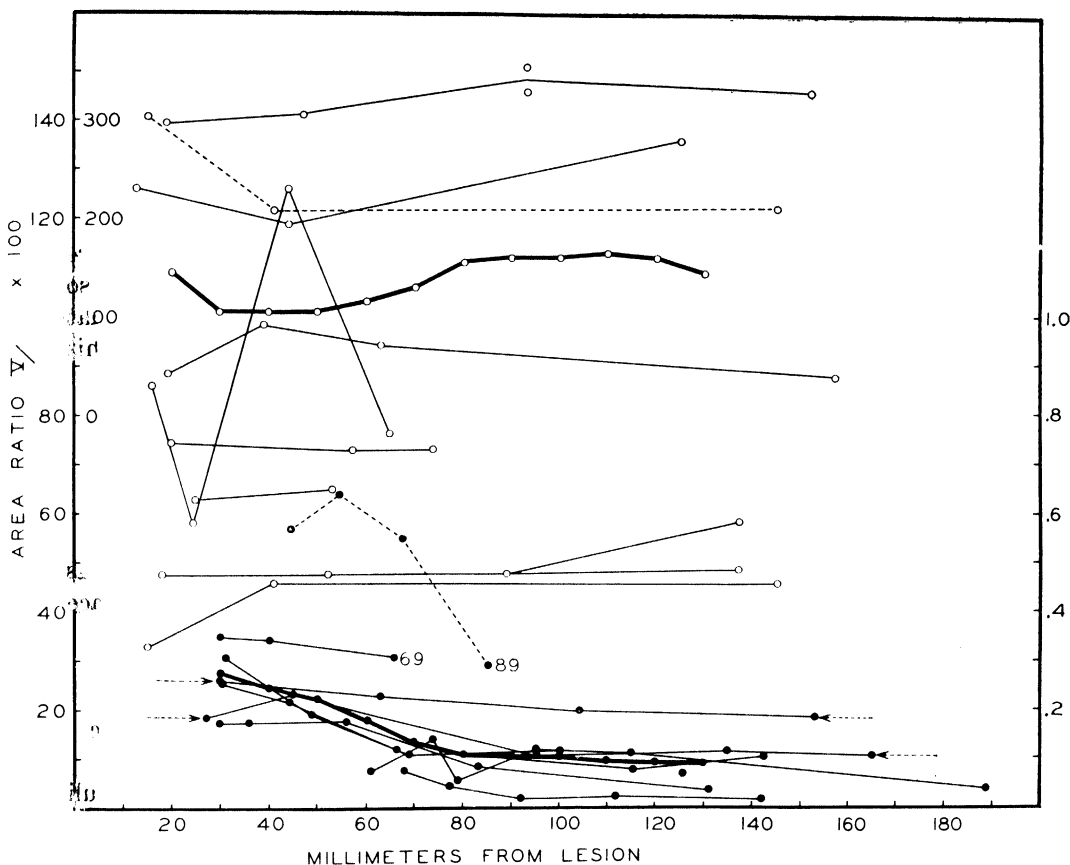


Fig. 6. Area ratios against distance from lesion.

Curves from normal preparations are designated by rings. The ordinate values for the broken line curve are inside the frame.

Curves from degenerating preparations are designated by dots. All are from 72 hour preparations excepting a 69 hour and an 89 hour preparation, as indicated. Ordinate values for the latter are to the right.

The heavy lines are the curves of the means.

Degenerating nerves. The curves of the area ratios obtained from seven 72 hour nerves, from a 69 hour nerve (indicated by the numeral 69) and from the 89 hour nerve, all indicated by dots, differ very decidedly from those derived from the normal nerves in that they fall lower in the graph and have a decided downward slope. The heavy curve is the mean of all of the data excepting those from the 89 hour preparation. It falls relatively rapidly from 27.5 at 30 mm. to 11

at 80 mm., i.e., through a range of 50 mm. situated between distances 30 and 80 mm. from the lesion. The fall continues thereafter but much more slowly. However, the number of observations beyond 130 mm. is not sufficient to supply a reasonable average.

The 89 hour curve (the broken line marked by dots,—ordinates to the right) lies far below the curves from the 72 hour preparations. The amplification factors in this case were to each other as 1:44, whereas the greatest amplification ratio for the 72 hour records was of the order of 1:10. Like the others, this curve slopes downwards. After 4 days we have not been able to detect any action potential at any point on the nerve within the range we have investigated,—i.e., out to a distance of over 200 mm. beyond the lesion.

Obviously, 72 hours after dividing the fibers only about one-fourth of the normal action potential, on the average, remains at a distance of 30 to 40 mm. from the lesion, and this fraction is further reduced as the distance from the lesion increases. After 89 hours there is a similar decline in area, but at a much lower level,—from 0.6 to 0.3.

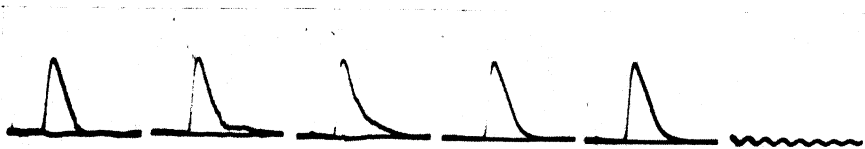


Fig. 7. Records (as photographed) from an experiment illustrating some difficulties encountered in ascertaining the behavior of the smaller fibers during degeneration. For key to records see table 1. Time in msec.

The foregoing observations on excitability, conduction rate and area supply data derived primarily from the larger fibers of the preparations. Now reference should be made to the difficulties encountered in attempts to ascertain how *the small fibers behave during degeneration*. This can be done best by describing one of the experiments designed to acquire information relative to this question. The preparation was one in which the Vth root fibers had been degenerating 72 hours. The nerve was stimulated with shocks increasing in strength in steps from "just maximal" to twice maximal. Two records, one at each extreme, were obtained from the normal (Vith) fibers and six, one at each of as many stimulation strengths, from the degenerating (Vth) fibers. Amplification was adjusted at the start of each of the two series so that the spikes of V and VI had approximately the same height when stimulation was just maximal. The relative amplifications required to accomplish this were 4 for V as compared with 1 for VI. The results are exhibited in figure 7 and in table 1.

During the process there was an insignificant increase in the heights of the spikes of both V and VI (see table 1) and a decided increase in the area of V,—from 156 to 193. The increase in the area of V was due almost entirely to the

addition of a trailing area to the "just maximal" response (see fig. 7). But the "just maximal" spike of V is a bit narrower than that of VI and this might be taken to indicate that some of the slower fibers normally contributing to it have become less excitable and slower still, which, if true, would account for the difference in the pictures derived from V and VI in this experiment. The Vth root, however, is normally the smaller of the two, and would therefore have the narrower fiber size range and the narrower spike. There are additional reasons for excluding slowing of the conduction rate in the slower fibers of the spike as the source of the potential for the tail that develops here on the spike of V, namely, (1) the area of the tail is far greater than the difference in the areas of the "just maximal" spikes of V and VI, and (2) the time to maximum of the crest of the action potential is the same for both V and VI. Other possible explanations of the development of the tail on V are *a*, that the Vth root, but not the VIth, normally carries a group of small fibers (which certainly is not the rule), and *b*, that the degenerating fibers in this case are repeating. However, repeti-

TABLE 1
Effect of shock strength on spike area

V				VI			
Record no.	Shock strength	Spike height	Spike area	Record no.	Shock strength	Spike height	Spike area
	% of maximal				% of maximal		
1	100.0	23.0	156	4	100.0	21.5	180
	112.5	23.3	156				
	125.0	23.8	171				
2	137.5	24.2	184	5	200.0	25.0	185
	150.0	24.8	180				
3	200.0	24.6	193				

"Record no." refers to the records, left to right, reproduced in figure 7.

tion, likewise, is not the rule under these circumstances;—we have many observations in which increasing the strength of stimulation beyond "just maximal" did not alter the shape of the action potential from the degenerating root. Indeed it has been the rule to find in degeneration experiments that the starts and the ends of comparable pairs of spikes are superimposable. Therefore we feel that the evidence does not justify the conclusion that during degeneration the slower fibers are affected first and become still slower.

Observations after interruption of all phrenic fibers. Since the foregoing controlled method for studying degeneration cannot be adapted to observations in which the lead is from the peripheral end of the nerve, several experiments have been performed in which all of the fibers entering into the phrenic were interrupted in the neck by crushing the Vth and VIth roots, and by cutting the entire VIIth cervical nerve, all at the same level. Area and conduction rate determinations were made *a*, with the lead fixed at the peripheral end of the nerve while shifting the position of the stimulator, and for purposes of comparison;

b, with the lead on the central end of the nerve while shifting the position of the stimulator. In this type of experiment the effects of temperature variations cannot be as perfectly eliminated as with the controlled method; moreover, comparative excitability determinations would be of little value and were not made.

Results. The results obtained in such an experiment are plotted in figures 8 and 9. With the normal nerve, when the lead was central (fig. 8, solid triangles,

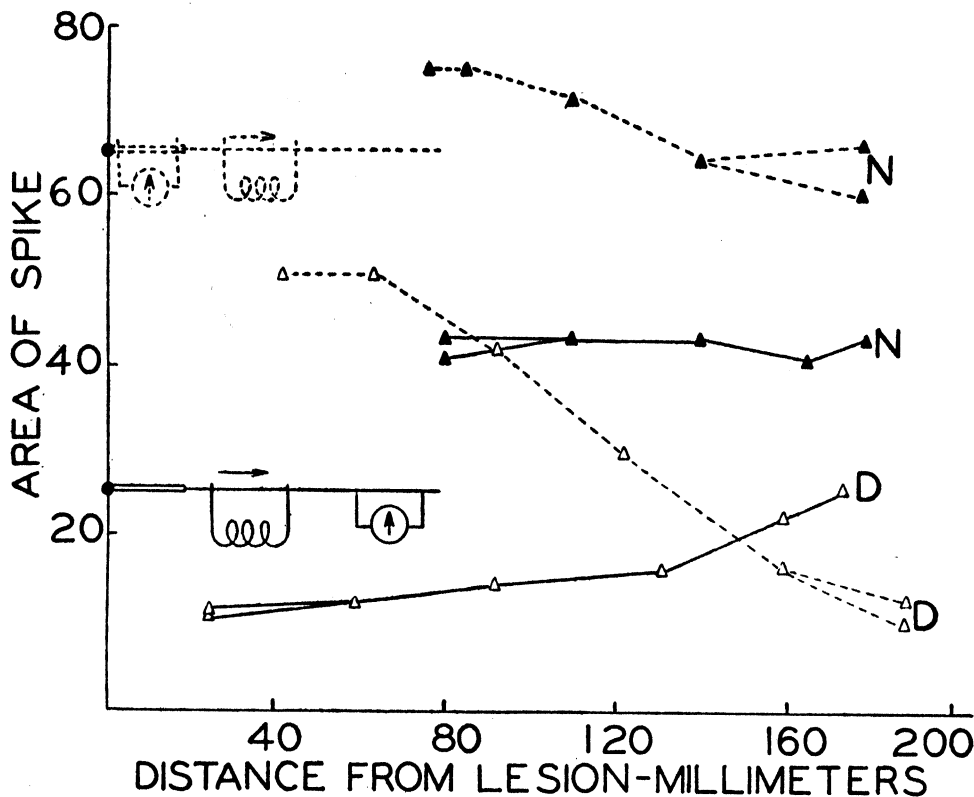


Fig. 8. Area of spikes from all the active fibers of the phrenic nerve *vs.* distance of conduction.

Dotted curves: lead central; line curves: lead peripheral.

Solid triangles: normal nerve; open triangles: nerve degenerating 76 hours.

dotted line) the area fell off slightly as the distance of the stimulator from the fixed lead was increased; the decline is at the average rate of 1.6 per cent per 10 mm. of shift. When the lead was peripheral (solid triangles, continuous line) the area remained essentially constant. Presumably these two results are the expression of the loss of fibers via branches to thoracic structures.²

² See Morris (1942).

With the companion nerve that had been *degenerating 76 hours*, when the lead was central (open triangles, dotted line) the *area*, as in the experiments by the ratio method, was low and fell off markedly with the distance. The rate of decline here averages 24.5 per cent per 10 mm. of shift. Deducting the 1.6 per cent average rate of decline exhibited by the normal nerve when the lead likewise

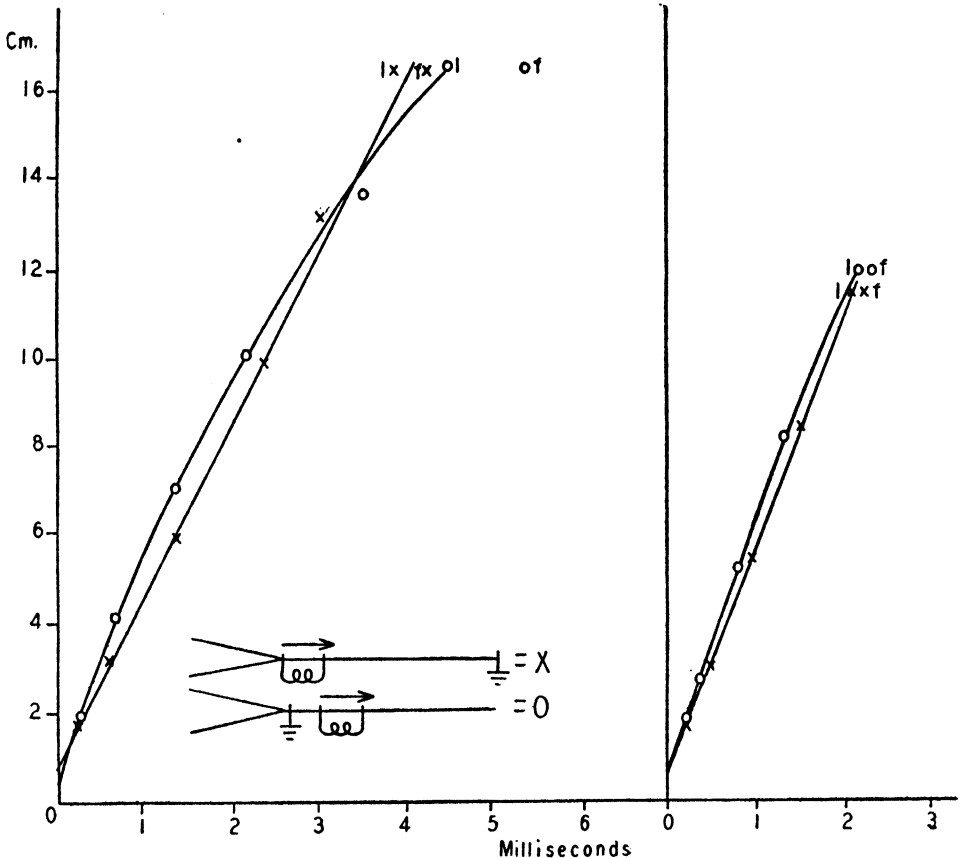


Fig. 9. Curves of conduction time vs. distance, derived from the nerves of figure 8. Circles: lead central; crosses: lead distal. *f* is the first determination of each series; *l* is the last determination of each series. (These letters are alongside the terminal circles and crosses.) Right hand graph: normal; left hand graph: all fibers degenerating 76 hours.

was central, the rate of decline here becomes 22.9 per cent per 10 mm. When the lead was peripheral and the distance from the lesion was increased, the *area* increased at the average rate of 8.5 per cent per 10 mm. through a length of 147 mm. However, more than half of this increase developed in the distal 45 mm. of the preparation; through the first 102 mm. the increase in area was practically linear and at the slow rate of 2.8 per cent per 10 mm.

The results of the conduction rate determinations are plotted in figure 9, those from the normal nerve in the right half of the figure, those from the degenerating nerve in the left half. In both graphs the circles give the results when the leads were on the proximal ends of the preparations, the crosses when the leads were on the distal ends. The sequence of the procedures was as follows. Both of the phrenics were excised at the same time. The degenerating nerve was mounted first, the normal nerve meanwhile being kept in the refrigerator. The nerve chamber was opened between each of the two sets of observations on a nerve in order to reverse its direction on the electrodes. The order of the observations in each case was from longest distance of conduction in steps to the shortest distance and then back in one step to the longest distance again. In each of the four series the first and the last observations are indicated in the graphs by the letters *f* and *l* (q.v.).

The order of the procedures is mentioned because apparently it influenced somewhat the results obtained. Thus it will be noted that the first determination of each of the four sets yielded a longer conduction time than the last, though the same full length of the nerve was traversed in each case. We believe that this result is a manifestation of temperature,—that each of the first determinations was made before the nerve and chamber, cooled by evaporation during manipulations, had quite regained equilibrium temperature. That equilibrium was re-established by the time the second determination of each of the series was made is supported by the fact that conduction in both of the curves indicated by the crosses, i.e., with lead at the peripheral end, is linear if the first determination at the longest distance be disregarded. For these reasons we feel that the *f* (first) points on each of the four curves can be disregarded.

Granting this, the results obtained are as follows: *a*. When the lead was from the peripheral ends the conduction rates were linear in both the normal and degenerating nerves but slower in the degenerating preparation. *b*. When the lead was from their central ends the rate fell off slightly as the stimulator approached the distal end of the normal preparation, but earlier and eventually more widely in the case of the degenerating preparation; and in the more proximal reaches the conduction rates in the two preparations were essentially alike.

Discussion. In this experiment, since the amplification was not altered, the recorded amplitudes decreased with increasing distance of conduction. The possibility has to be entertained, therefore, that the departure of the conduction rate from linearity when the lead was central was due to error in the measurement of the conduction times. There are, however, reasons for excluding this as a probability. Thus the changes in amplitude in both of the curves from the degenerating nerve were such as would produce an error in the same direction, though, to be sure, not of the same magnitude. Thus with lead central (circles) the amplitude changes ranged between 1 and 8.1, and with lead peripheral (crosses) between 1 and 4.45; yet one of the curves, the latter, is quite linear, whereas the other is very decidedly curved. Moreover, with the normal nerve the amplitude range was between 1 and 1.5 with conduction in either direction; and yet the curve when the lead was peripheral, is linear whereas with the lead

central it is not. It follows, therefore, that the departure from linearity with lead central is not due to any error attributable to amplitude changes. There is left as an explanation of the behavior of the conduction rates in this experiment an effect of the number of fibers conducting to the lead. But the further development of this explanation requires a diagram which will be considered in a subsequent section.

In four of five additional experiments of this kind the increase in area as to stimulator approached the lead on the distal end of the nerve amounted, in per cent per 10 mm. shift of the stimulator, to 0, 0.5, 1.3 and 1.8. It is important to note that where there are increases they are insignificant; that, moreover, there was in these cases no acceleration of the increase in area as the stimulator approached the lead on the distal end. The fifth preparation was damaged at its proximal end and is regarded as unsatisfactory.

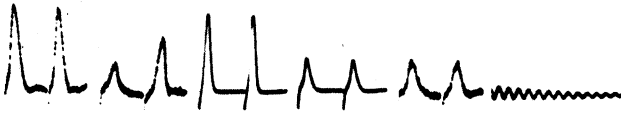


Fig. 10. Selected records (reproduced in photographed size) from a fatigue experiment. Rate of stimulation 213 per second.

1st pair of spikes: From degenerating fibers (72 hours) at start of stimulation.

2nd pair of spikes: From degenerating fibers after about 95,000 stimulations.

3rd pair of spikes: From normal fibers at start of stimulation.

4th pair of spikes: From normal fibers after about 95,000 stimulations.

5th pair of spikes: From the degenerating fibers after about 130,000 additional stronger stimulations.

6th record: Time in msec.

The relative fatigability of normal and degenerating fibers (72 hrs.) has been determined in 5 preparations. Description of a typical experiment will serve to indicate methods employed and results obtained. The stimulating electrodes were on the nerve trunk so that both sets of fibers were stimulated simultaneously. The rate of stimulation was 213 per sec. Amplification was adjusted so that initially the spikes of both sets, led from the roots separately, were approximately alike; it was as 1 is to 26 for the normal and the degenerating fibers, respectively. The action potentials were led first from the Vth root, using a stimulation strength that sufficed to eliminate alternation of spike height. But after about a minute of stimulation alternation reappeared. So, after a long rest period, and after setting the stimulation strength "well above maximal", stimulation was resumed and was continued for 2 minutes while taking records (see fig. 10). Then, after further increasing the shock strength and after a rest period of 12 minutes, stimulation was resumed and records were taken from VI for a period of about 2 minutes. And finally, without stopping stimulation or

changing any of the conditions, records were again taken from V. This alternation of procedure was necessary because the means for recording simultaneously from the two sets of fibers were not available.

In figure 11 the data from the second, third and fourth series are plotted, amplitude of spike in per cent of the height of the first against the number of stimuli. A better measure would have been the areas of the spikes, but inspection of the records (see fig. 10) will show how inaccurate this would have been, and that it would have altered immaterially the essential features of the graph. The graph shows that the spike heights fall off rapidly at first,—that after about

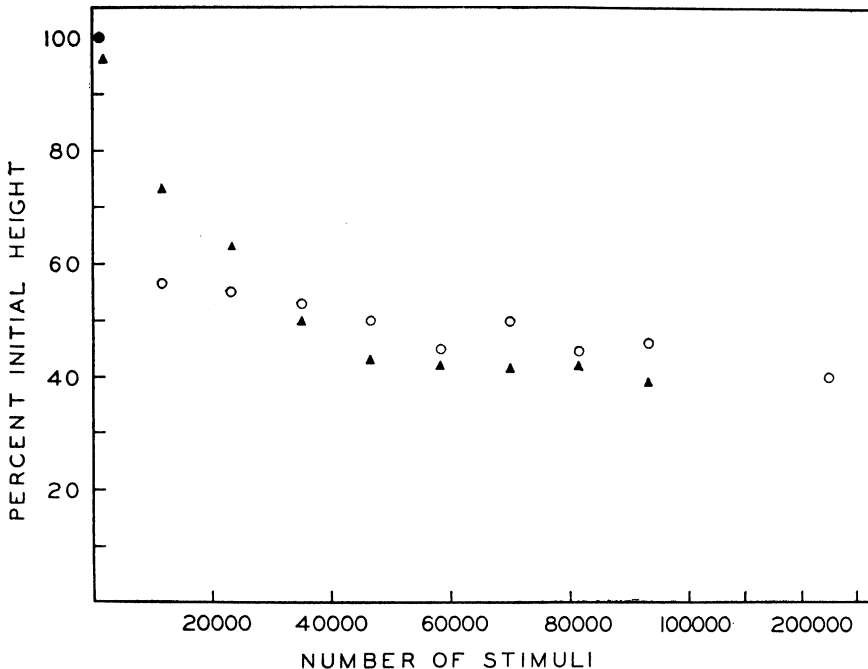


Fig. 11. Graph of spike height in per cent of first response, against the number of stimuli,—rate 213 per sec.

Triangles: responses from normal fibers.

Circles: responses from fibers degenerating 72 hours.

40,000 responses there is little further reduction in height,—and that the relative heights of V and VI change in like manner. The records, however, show a decided difference in the behavior of the responses of the two sets of fibers, in that those from V begin to alternate in height some time before the 10,000th stimulation, an alternation that increases throughout this phase of the experiment. The heights accepted for the plotting of V when it was alternating are the averages of two successive spikes; the fluctuations of the Y values of V are an expression of this method of procedure. The last point on the graph gives the height of the spike of V after more than 130,000 additional responses. Now,

with the stronger stimulation used in this part of the experiment, alternation is practically absent (see fig. 10) despite the protracted stimulation.

Alternation of spike height of V developed in two other experiments. In the remaining two there was no alternation, though the conditions obtaining in all of these experiments were essentially alike. In one of the latter two experiments the heights of the spikes from the normal and degenerating fibers declined 50 and 25 per cent, respectively; in the other experiment the heights declined 36 and 32 per cent, respectively. In these two experiments, therefore, there was no consistent difference in the fatigability of the two sets of fibers.

Discussion. The tendency for the spikes of the degenerating fibers to alternate presumably is the expression either of an initially relatively long recovery period, or of a greater effect of fatigue on the recovery period of degenerating fibers as compared with that of normal fibers. Possibly it is related to the finding that the half-maximal excitability of the degenerating fibers may be a trifle lower than that of the normal fibers.

Titeca (1935) finds, in experiments performed apparently on frogs only, "a very striking exaggeration of fatigability" appearing relatively early in degeneration and developing centrifugally. His stimulation rates commonly were about 50 to 60 per sec. The temperatures at which he worked are not specified, excepting the statement that during degeneration the frogs were kept at 19° to 20°C. When these conditions are compared with ours, namely, stimulation rates of over 200 per sec. and working temperatures of about 26°C, it is obvious that our conditions were far more rigorous than were his. And whereas our experiments do disclose some increased fatigability in degenerating fibers, as evidenced in some of the preparations by the tendency toward alternation, the increase is slight almost to the vanishing point. If the motor fibers of the phrenic nerve had the properties of our 72 hour fibers fatigue would never become a factor in their functioning at body temperature and at the physiological rate of perhaps 50 impulses per second.

It may be of interest to note that the relatively refractory period is concerned with recovery, the spike with activity. Since the latter is scarcely altered during degeneration whereas the former is changed, though slightly, these findings may signify that the recovery process is affected more during degeneration than the active process.

General discussion. The significance of the data on area relative to the question of the progression of degeneration can be seen in the diagrams of figure 12. Three possibilities are represented, namely, from above downward, *a*, centrifugal and *b*, centripetal degeneration and *c*, conduction failure at loci randomly scattered along the fibers, but statistically less widely spaced peripheralwards. It is accepted that axon action potentials and conduction rates are not materially altered.

Each of the three groups of parallel lines diagrams a degenerating nerve, the lines, including the boundary lines, representing the parts of the constituent fibers that still react normally. In the third group the interruptions represent non-conducting blocks. The lesion, represented by dots, in all cases is at the central,

the left, end. The series of graphs on the right and left sides depict schematically *a*, by the "line" curves only, the results anticipated on the basis of the indicated procedures, and *b*, by the curves indicated by the dots only, the results actually obtained experimentally. Of the "actually obtained curves" (dots), the one with the lead central (left hand graphs) was derived from the curve of averages obtained through the use of the controlled method (see fig. 6) in the stretch subtended between the distances 30 and 130 mm. by fitting them, in their proper proportions, into the co-ordinates of the diagram.

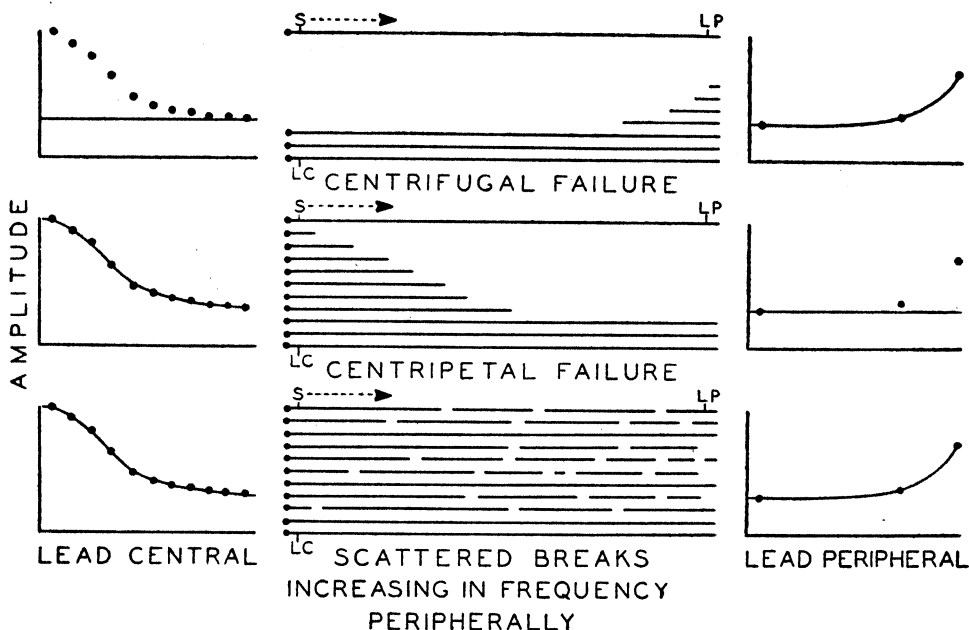


Fig. 12. Diagram of the anticipated area changes with lead central *L.C.*, or lead peripheral, *L.P.*, as the stimulator, *S*, is moved away from the lesion (to the left), if degeneration proceeded centrifugally (top), centripetally (middle) or developed through scattered interruptions increasing in frequency peripheralwards (bottom). For further explanation see text.

The available data when the lead was peripheral were obtained under such varying conditions that it is not possible to derive from them a curve of averages. It has been seen, however, that those curves in general slope upwards very slightly, if at all, through the whole or through a considerable part of the available lengths of nerve, but that, in the latter cases, the curves may finally bend upwards. The latter result is outlined by the dots in the diagrammatic graphs on the right side (lead peripheral).

It thus is made obvious that failure *does not proceed centrifugally*; that a fit between the predicted and the actual results is obtained only on the assumption that *conduction fails at scattered loci increasing in frequency peripheralwards*. It is obvious, however, that an increase in the frequency of the scattered peripheral

blocks would increase the flatness of the area curves that are derived through a peripheral lead and thus give a set of curves corresponding best with the picture of centripetal failure.

All of our relevant observations support the conclusion that the first evidences of failure appear in the more distal stretches of the nerve. Thus if there develops during degeneration any gradient in conduction rate or in excitability it is in the direction of a slight peripheralwards decrease. And there is no question but that the area, as determined by our comparative method, falls off peripheralwards. This conclusion, moreover, is supported by the results of the experiment described in a preceding section in which all of the fibers of the phrenic were degenerating. That experiment indicates, in addition, that such slowing of conduction and reduction of excitability as there may be possibly are due not to conduction and excitability changes *per se*, but rather to the abrupt failure of conduction before any other changes in reactivity become apparent.

In picking up the thread of the discussion of that experiment where it was broken, a way of accounting for the behavior of the conduction velocity under the different conditions will now be considered. The velocity we determine is that of the largest fibers. On a statistical basis the larger the total number of fibers the wider will be the range of fiber sizes and consequently the higher the velocity. In the experiments of the type now under consideration, dealing, as they do, with all of the fibers of the phrenic, the number of fibers concerned is far greater than was the case when the fibers of only a root contributed to the spikes. Moreover, in the present experiment the percentile diminution of spike area in the degenerating nerve was many times greater than in any of the experiments in which root potentials were recorded. Therefore any effect that fiber size range might have on conduction rate should be more apt to become evident in the present experiment than in the root experiments, in which, it should be stated, an effect of the number of responding fibers on the conduction velocity was not obvious.

On the basis of these considerations one might expect to find in the *normal, whole phrenic preparation* with lead peripheral (crosses) a linear conduction rate that was slightly slower than the conduction rate obtaining with lead central (circles), and in the latter case perhaps some slowing of the conduction velocity at the longer reaches. The results, plotted in the right hand graph of figure 9, show that this is the case.

In the preparation *all* of whose *fibers* are *degenerating* the number conducting to the peripheral lead would increase markedly as the distance decreased if degeneration proceeded as indicated in the first diagram of figure 12, and an increase in conduction rate might be expected; but, as a matter of fact, conduction is linear (fig. 9, left hand graph, crosses). If degeneration proceeded according to the middle scheme, the number of fibers would remain constant under the procedure and would be small; the rate might therefore be expected to be constant but slower than in the normal preparation, and this is the case. And according to the third scheme, the number of fibers might perhaps increase slightly at the shorter distances, and the rate might therefore be expected to increase

slightly but be slower than in the normal preparation. As a matter of fact the rate under these circumstances is linear in both the normal and the degenerating preparations; but it is faster in the normal preparations, namely, 54.6 mps. as compared with 42.2 mps.

Finally, with lead central the number of fibers responding would, according to scheme 1, remain small and constant as the distance of conduction decreased and the conduction rate should be slow and linear; according to scheme 2 the number of fibers would increase to a relatively large figure and the conduction rate would be expected to increase; and finally according to scheme 3 the conduction rate should increase, but somewhat less rapidly, at the shorter distances. Figure 9 shows a definite increase in conduction rate, particularly at the longer distances, as the conduction distance shortens.

We conclude, therefore, that the behavior of the conduction rate is in general consistent with the predictions based on the second and third diagrams and on the assumption that fibers fail at a time when conduction and excitability still are normal in those fibers that continue to conduct to the lead. The behavior is wholly inconsistent with centrifugal failure. That conduction fails at a time when reactivity still is essentially normal and that it fails more or less suddenly, has been noted by other investigators (e.g., Titeca, 1935; Holobut and Jalowy, 1936; Rosenblueth and Dempsey, 1939).

The histological studies of Weddell and Glees on nerve degeneration (1941), when considered in the light of what is known regarding the period through which voluntary muscle continues to respond to indirect stimulation of its severed nerve supply, we believe, a plausible explanation of the fact that at a time when, according to our observations, say, 75 per cent of the fibers no longer conduct the reactivity of those that still conduct remains practically normal. Using the response of muscle to indirect stimulation as an index, Holobut and Jalowy have found that contraction does not fail after nerve section until 3 to 5 days have elapsed. The observations of Weddell and Glees were made on fibers (they were in the skin) which probably do not differ materially in size from the terminal motor fibers. According to them, *a*, histological changes become obvious *within 12 hours* after section, at which time "less than one-third of the *fibers of all diameters* (italics ours) are indistinguishable from the normal"; and *b*, at 48 hours "all nerve fibers . . . show signs of degeneration in some part of their course." The axis cylinders exhibit the earliest signs of degeneration, but after 96 hours of degeneration the majority of the myelinated fibers are in the form of "digestive chambers." To form these digestive chambers, or illipoids, the myelin breaks into droplets of various sizes. In contrast with this schedule, we find that *at 72 hours* as many as 25 per cent of the fibers still may be responding almost, if not quite, normally; that even at 90 hours the 0.6 per cent of fibers that still respond are practically normally reactive, at least in their more proximal stretches. These observations, together with the fact that muscles cease to respond to indirect stimulation only after a lapse of from 3 to 5 days, can only mean that the histological changes that develop in the axis cylinder prior to that time have little or no effect on the reactivity of nerve. In the premises it seems necessary to conclude that despite definite and even marked histological altera-

tion in the axoplasm, the surface membrane of the axis cylinder continues to function normally as long as it has the support of the myelin sheath; and that conduction ceases only with the disruption of the myelin sheath. Possibly what then happens is that the electrotonic current spreading ahead of the active locus is shunted by the low resistance at the breaks in the myelin and consequently no longer attains a strength that is sufficient to restimulate the fiber beyond.

Weddell and Glees find that in degenerating fibers the lesions increase in frequency distally and are more marked in the smaller fibers. A peripheralwards increase in the frequency of abrupt blocks such as breaks in the myelin might cause, would account quite satisfactorily for the conclusion we have reached that nonconducting loci increasing in frequency peripheralwards account for our results. We do not, however, have any clear evidence indicating that small fibers fail before large.

Holobut and Jalowy, using rat's nerve-muscle preparations, also describe marked histological degeneration at a time (after 24 hrs.) when excitability, using the muscle as the index, is within normal limits. Though they state (p. 548) that the histological changes begin and develop simultaneously along the entire length of the peripheral section, they conclude that fragmentation in the peripheral regions causes an interruption of the connection between nerve and muscle, a statement which seems to imply that in motor fibers, too, the peripheral regions disintegrate before the more central regions.

It is well known that in neuritis due to chemical agents it is the longer nerve fibers that first cease to function. Does this signify that failure resulting from chemical agents proceeds centripetally also?

The question now arises, is there any way of accounting for results of those (Rosenblueth *et al.* and Parker, e.g.) who have concluded that degeneration proceeds centrifugally. The only suggestion that occurs to us is that their procedures disturbed the blood supply to the degenerating nerve. Bentley and Schlapp (1943) have shown that a small but definite blood supply is essential for the maintenance of conduction in the politeal of the cat. And it may be possible that although such interference with blood supply as is caused by the section of a nerve does not result in any alteration in its ability to respond during the course of 6 to 8 hours, it may nevertheless influence the resulting progression of degeneration, even when, as in Rosenblueth and del Pozo's experiments, care may have been taken not to cut the arteries which accompany the nerve. It so happens that the part of the phrenic subjected to our experimental procedures receives its blood from the mammary artery; therefore the preliminary operation (crushing the roots in the neck) would not appreciably alter the blood supply of the preparations we use.

Parker's (1933) conclusion that degeneration proceeds centrifugally in the frog's sciatic is conditioned not only upon a possible reaction to disturbed blood supply, but also upon the employment of a stimulus strength that was maximal at all points on the nerve. For example, if the adjustment to maximal was made on the tibial or peroneal nerve there is the possibility that stimulation might have been submaximal with respect to the plexus at the hip level.

Finally, it does not seem possible that we have failed to find peripheralwards

degeneration because in our experiments the first 30 to 40 mm. of the nerve beyond the lesion were not available for study by the comparative method. It does not seem possible because practically all investigators have employed lengths up to 80 mm.

REGENERATION. General Method. The routine procedure in the study of regeneration by the comparative method has been to crush the Vth root in the neck as far aborally as conveniently possible, marking the locus with a loosely tied black ligature. At appropriate intervals thereafter the phrenic nerve was excised along with the Vth and VIth roots and mounted as were the nerves in the experiments dealing with degeneration. In what may be designated as the old series (O.S.) of experiments, eleven in number, with regeneration times ranging between 29 and 127 days, the lead from the regenerating root was from a point a few millimeters central to the lesion when the regeneration time was short, and a few millimeters peripheralwards of the lesion when the regeneration time was long enough to permit of satisfactory recording directly from the regenerating fibers. In what we designated as the new series (N.S.), constituting twelve successful experiments with regeneration times ranging between 31 and 343 days, the lead from the regenerating root was always a few millimeters central to the lesion.

Results again are expressed as ratios. In this case, however, the ratios are $\frac{\text{response from regenerating root (VI)}}{\text{response from the normal root (V)}}$ usually $\times 100$, since the control root in this series is the Vth, not the VIth, as was the case in the experiments on degeneration. And since the VIth root is usually the larger of the two, the normal ratios for this series should exceed 100 slightly. Conclusions are based for the most part on data derived from the new series.

Excitability. For the determination of excitability the half-maximal method was used. The data from the new series of experiments are plotted in figure 13, the excitability ratios against the distance of the stimulator from the lesion. The figure shows:

- (1) That in general the excitability increases with regeneration time.
- (2) That even after 343 days the ratio, ranging as it then does around 45, still is far below the normal value of 100+.
- (3) That despite some irregularity, the excitability obviously declines with the distance from the lesion through some 114 to 130 days. In the more proximal reaches the rate of decline seems to increase with regeneration time to attain a maximum after about 40 to 60 days. The rate of the decline with the distance then diminishes and the curves become approximately horizontal.
- (4) In the three youngest nerves (29 and 30 days, O.S., and 31 days, N.S.) the excitability ratios at the proximal loci are less than 5; at the most distal loci they do not exceed 3, and may be as low as 1.

The significance of the intermediate stage of most rapid decline of excitability with the distance is not apparent. Possibly it is related in some way to the laying down of myelin.

In the earlier stages of regeneration it was not possible to make *chronaxie*

determinations, owing to the inadequacy of the apparatus available for the purpose. Determinations became possible only when the excitability had risen to the level obtaining in the 343 day preparation. The chronaxie of the most excitable fibers of both sets, normal and regenerating, then lay between 0.08 and 0.10 msec.; in other words they had the same excitation times. Yet at this stage the excitability of the regenerating fibers as determined by the half-maximal method still is less than 50 per cent of the normal. Further evidence is thus made available which indicates that rheobase may be independent of excitation-time parameters (see Davis and Forbes, 1936). In this connection it may be recalled that these same time parameters are associated with nerve impedance

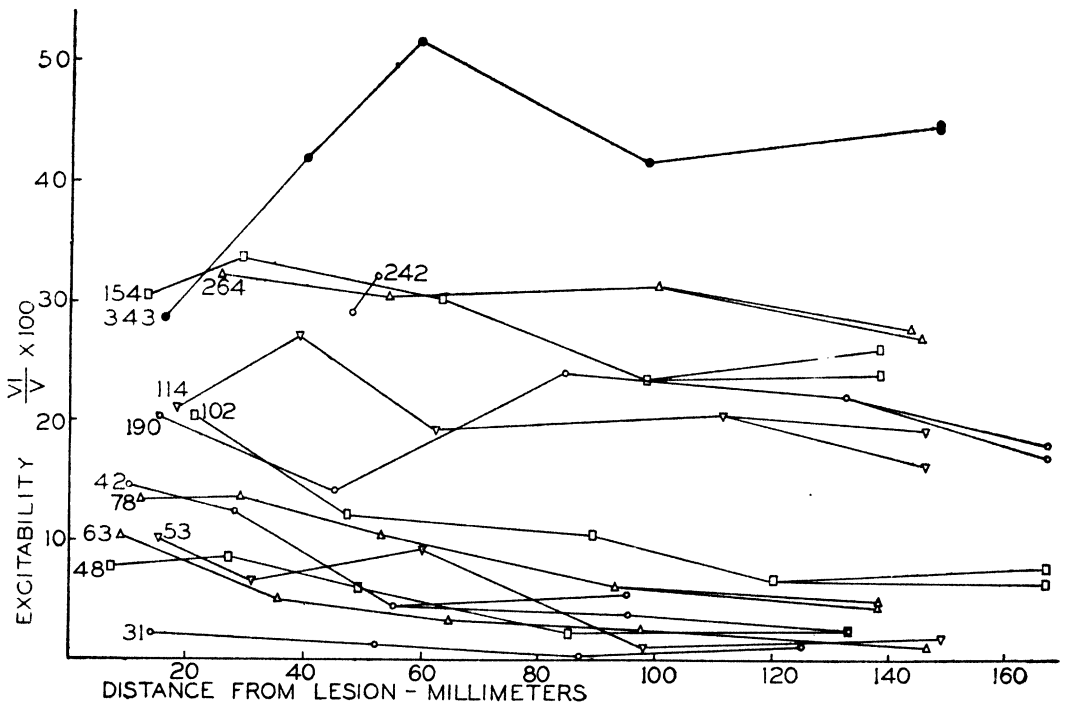


Fig. 13. Excitability ratios *vs.* distance from lesion. The numerals give regeneration times in days.

(see Schoepfle, 1944). The overall impedance as manifested in the time course of the electrotonic potentials is known to be independent of nerve viability. Hence a change in threshold of the excitable structure proper need not necessarily involve the nerve impedance to the extent of altering the time parameters.

On the basis of fiber size measurements of others (Gutmann and Sanders, 1943), the two sets of fibers at this stage of regeneration, i.e., 343 days, have the same diameters and should be equally excitable; yet, as just stated, the regenerating fibers by the half-maximal method have less than half the excitability of the normal fibers. This situation may signify that the regenerating fibers are less

accessible to applied currents than the normal fibers. Thickened fiber sheaths rather than altered "excitable structures" could account for this situation.³

Conduction Rate. Methods. In most cases, as has been stated, the lead from the regenerating roots has been from a point a few millimeters central to the lesion. It is known (Gutmann and Sanders) that the diameters of the central fibers diminish for at least 130 days and then increase to attain their normal sizes after 250 to 300 days. It has not been possible for us to make an accurate allowance for this size behavior. The best we have been able to do in calculating conduction rates has been to assume that in the few millimeters of conduction central to the lesion the rate was the same as in the fibers of the normal root of the same preparation.

The *results* have been plotted in two ways, as was done in the degeneration experiments. In figure 14 the ratios of the conduction rates are plotted against

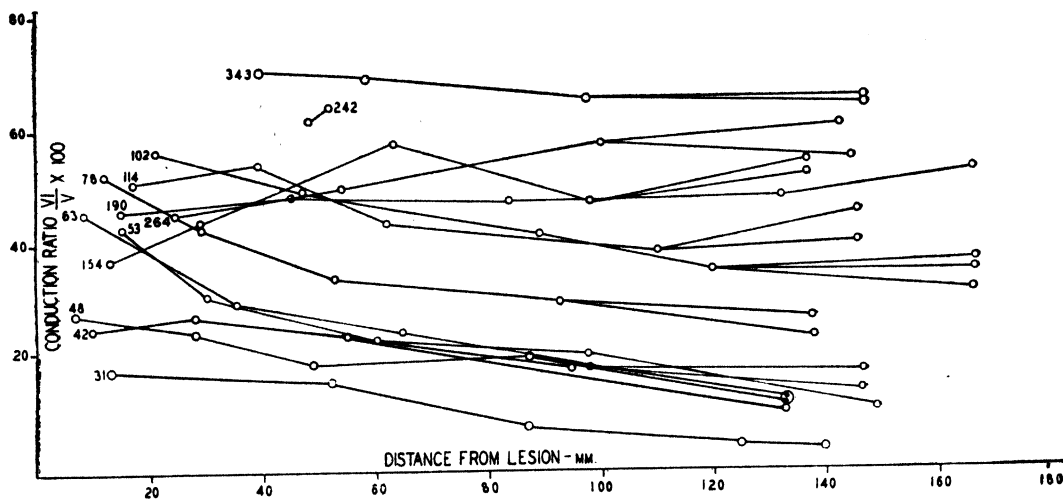


Fig. 14. Conduction rate ratios vs. distance from lesion. The numerals give regeneration times in days.

the distance from the lesion. This method permits of the presentation of the whole series in a single graph. The relative elevations of the curves indicate the rate of recovery of velocity with the passage of time. Despite the fortuitous effects of such factors as anatomical variation in the relative sizes of the roots, possible damage done the roots in preparation, etc., the increase in conduction rate with recovery time is quite obvious. The elevation of the several curves increases rapidly through about 154 days to the ratio of 50+, approximately. The further rise through the 189 additional days carries the ratio roughly to something less than 70. Between the 242nd and 343rd days there is practically no change.

The slopes of the curves of the ratios indicate the sign, but not clearly the magnitude, of the changes in conduction rate with the distance from the lesion,

³ Paul Weiss informs us that the Schwann sheath of a regenerating fiber is permanently thickened.

since by this method the total distance is divided by the total time. It is obvious, however, that in the early stages of regeneration the rate falls off much more rapidly in the regenerating fibers than does any rate gradient there may be in the normal fibers.

Graphs of the distance of conduction against conduction time give this information more clearly. In figure 15 are plotted the data from a young (31 day) preparation (circles), with time laid off below the graph, and from an older (264 day) preparation (triangles), with time indicated above the graph. The *normal fibers* of both preparations are conducting linearly and at approximately the same rates, namely, 42 and 41 mps., respectively. The *regenerating fibers* of the older preparation also are conducting linearly but at the rate of 22.5 mps. The conduction rate in the fibers regenerating for 31 days, however, falls off rapidly

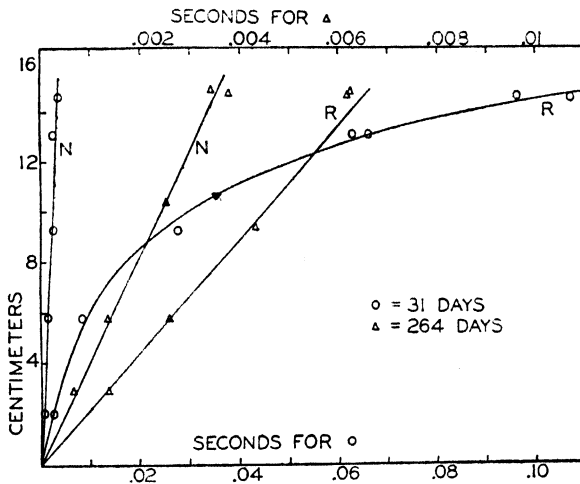


Fig. 15. Velocity curves in normal, *N*, and regenerating, *R*, fibers from two phrenic nerves in which the VIIth root fibers are regenerating, in one (circles) for 31 days, in the other (triangles) for 264 days.

with the distance: it is 7.4 mps. 20 mm. from the lesion and 0.8 mps. at 125 mm.; and the rate beyond that is slower still, but difficult to estimate.

All of the conduction rate data of the new series have been plotted similarly. The resulting graphs fit between and beyond the two of figure 15 to form a continuous series. The conduction rate in the regenerating fibers thus determined is approaching linearity at 114 days, and is nearly perfectly linear at 190 days. Linearity is achieved when the curves, as plotted in figure 14, become parallel to the base line.

Discussion. Berry *et al.* maintain, with some qualification, that the conduction velocity in regenerating fibers is linear at all stages; and Grundfest has remarked (1943) that conduction "is uniform all through the regenerating nerve fibers." They also maintain, or at least imply, that regenerating fibers have uniform diameters at all stages. This, however, is not in agreement with the histological

observations of Gutmann and Sanders, who find that the "size factors" of regenerating fibers decrease with the distance from the lesion (range from 10 to 80 mm.) and increase with the time through at least 250 days. Our conduction rates are approximately linear through that distance, namely, 80 mm. by 154 days.

Our conduction rates attain approximately 62 per cent of the normal after 343 days; possibly there would be some further increase with further time. Assuming that the normal rate of conduction in the experiments of Berry *et al.* is 110 mps., our calculation shows that their nerves recovered about 73 per cent of the normal in 343 days. In this respect our results agree fairly well with theirs.

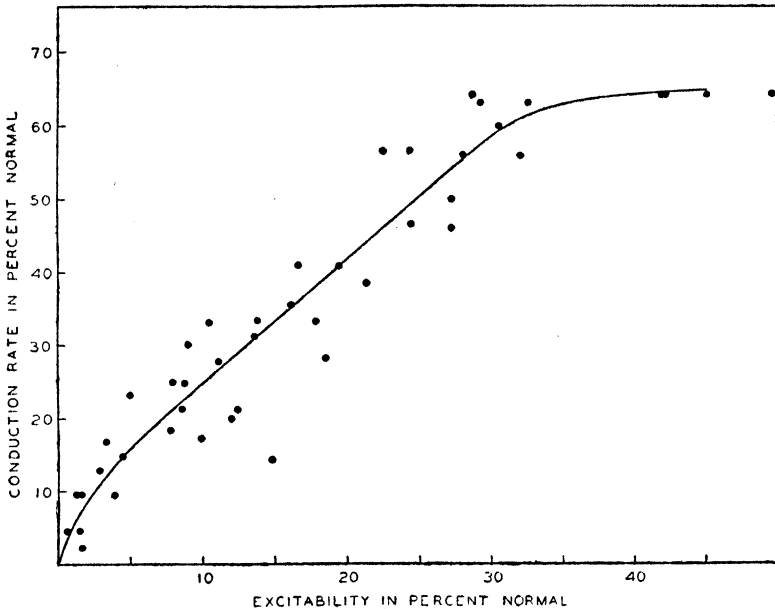


Fig. 16. Conduction rate *vs.* excitability, each in per cent of normal. All of the N.S. data are plotted.

Both their and our curves indicate that the conduction velocity will never return to normal.

The relation that obtains between conduction velocity and excitability is seen in figure 16, in which are plotted all of our determinations, with conduction rates of the regenerating fibers in per cent of normal as ordinates and the excitabilities in per cent of the normal as abscissas. The scattering of the points, though considerable, seems to be around a straight line which either intercepts the axis of the abscissas to the left of the origin or bends down to meet the origin, and possibly is becoming horizontal at its upper end. The curve seems to indicate that neither the excitability nor the conduction rate ever will attain normal values. The fact (see fig. 16) that the excitability, as determined by the half-maximal method, recovers less rapidly than the conduction rate and that the fiber sizes

ultimately become normal (Gutmann and Sanders) leads us to infer that the failure to return to normal is referable to the persistence of a fiber sheath of high resistance. Such a resistance would reduce the strength of the eddy currents which are believed to determine the propagation of the nerve impulse, and in this way slow conduction, despite the return of the diameter to normal.

Area Ratios are conditioned by a number of circumstances which complicate their interpretation. These include (1) all of the factors that determine the wide range of the levels of these ratio curves from normal nerves (see fig. 6), (2) variations in the number of fibers sending outgrowths into the distal nerve and (3) the size of the fibers central to the lesion, and this, through a considerable period of the regeneration time, will depend upon a , the regeneration time, and b , the distance of the lead electrode from the lesion. Therefore, for completely adequate information, averages would be needed of the results from a considerable number of nerves at each regeneration age and only occasionally do we have as many as two.

Results. The *relative elevations* of the area curves of figure 17, for reasons just mentioned, are without significance.

The *slopes of the curves* are better visualized in figure 18. Here the ordinates are the reciprocal of the height of the start of each of the curves of figure 17 (excepting that of the 242 day preparation) over the height of its end, times 100, and the abscissas the recovery time in days. This procedure is permissible because all of the curves are roughly of the same length. Horizontal curves would have a value of approximately 100 and would signify that all of the central fibers have extended their outgrowths to the distal end of the preparation; in other words, that "regeneration is complete". And smaller values give the per cent of central fibers that have failed to attain the end of the preparation.

For reasons given above, there is a wide scatter of the points. The indications are, however, that by 50 days fewer than half of the central fibers have grown the full length (roughly 140 to 160 mm.) of the preparation and that the number is approaching 100 per cent by, say, 100 days, though there is one aberrant result at 264 days.⁴

An approximation to the *fastest rate of progression of the fiber tips* is given by two preparations in which the action potentials from the regenerating fibers at highest amplification became illegible as the stimulator was moved distalwards. In a 29 day preparation this occurred at a distance of 115 mm. from the lesion, and in a 30 day nerve at 125 mm. Allowing 5.2 days for latency, the rates of growth thus determined are at least 4.83 and 4.84 mm. per day, respectively, a figure that exceeds slightly the value derived by Young (1942) (4.36 ± 0.24 mm.) by the reflex method in the rabbit.

Conduction through the Lesion. Three determinations have been made of the conduction time in either direction through the lesion on the root. For this purpose the two pairs of electrodes were fixed in position, one on either side of the lesion, with the proximal electrode (with respect to the lesion) of each of the

⁴ Such a result could be caused by any of the many fortuitous conditions interfering with the outgrowth of fibers.

pairs equidistant from the lesion. To change the direction of conduction the stimulating and recording connections were interchanged.

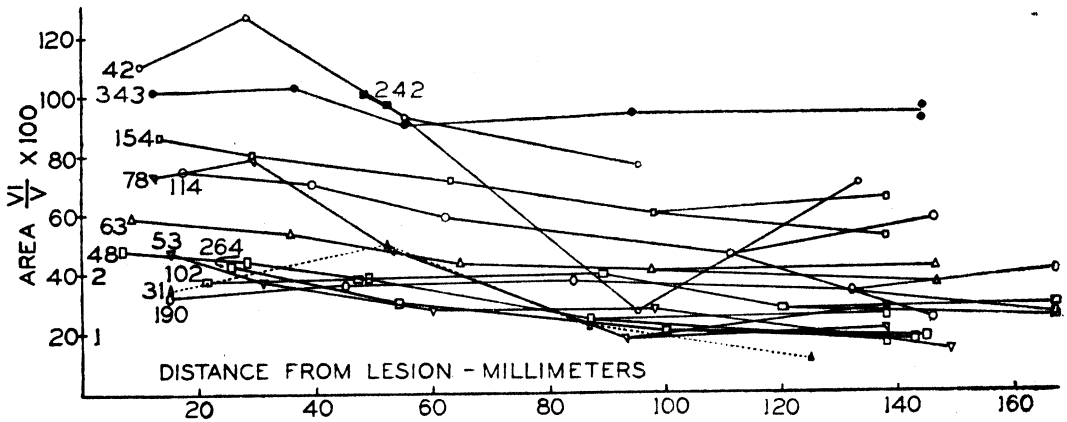


Fig. 17. Ratios of the areas of the action potentials vs. the distance of the stimulator from the lesion. The ordinates for the dotted, the 31 day, curve appear inside the vertical axis.

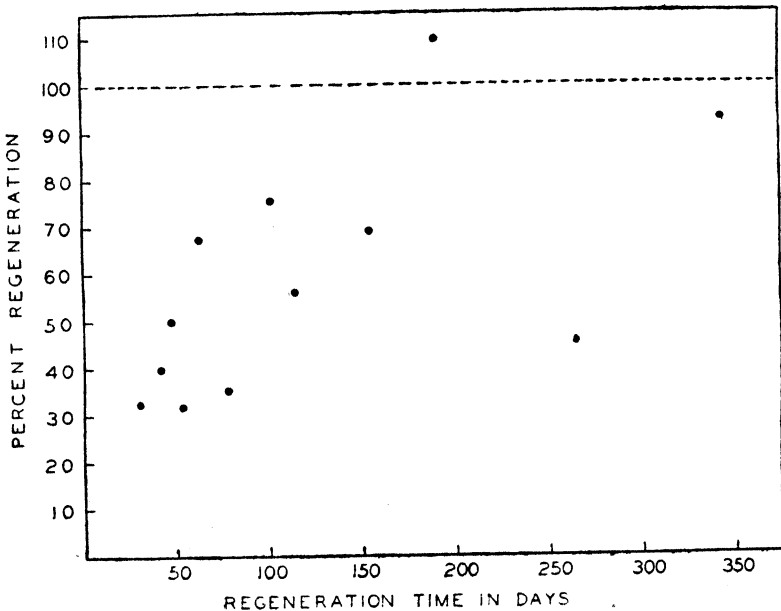


Fig. 18. Per cent regeneration. Ordinates, reciprocal of the ratio of the area of the action potential about 40 mm. from lesion over the area of action potential about 140 mm. from lesion $\times 100$. Abscissas, regeneration time in days.

The number of observations is not sufficient to justify any definite conclusions, but taken at their face value (see table 2), it would appear that early in regenera-

tion the transfer of impulses from normal to regenerating parts is less ready than in the reverse direction; that by 42 days the transfer occurs with equal facility in either direction. This observation, if confirmed, would signify that the impulse is transmitted with greater facility when passing from a region of low excitability to one of high excitability than in the reverse direction. This would be in keeping with the view expressed by Engelmann (1895).

Discussion. Young, in discussing the degree of recovery of sensation after nerve injury, concludes (1942, p. 349) that "the more delicate functions must . . . remain imperfect." In addition to other factors that might be responsible for this result he specifies failure of some of the severed fibers to regenerate and a persisting alteration in fiber size pattern. Now, since, as figure 18 indicates, every severed fiber can extend an outgrowth to the very end of the preparation, it follows that failure of some of the fibers to regenerate is not an inevitable consequence of "neurotomy." Moreover, it may be necessary to add to Young's

TABLE 2
Conduction time in either direction through the lesion

REGENERATION TIME	DIRECTION OF CONDUCTION	CONDUCTION TIME
<i>days</i>		<i>sec.</i>
29	Regen. to norm. Norm. to regen.	0.00268 0.00300-0.00325
42	?	0.00150 0.00178
109	Regen. to norm. Norm. to regen.	0.00047 0.00040

category two additional factors, namely, failure of excitability and of conduction velocity to return to normal.

SUMMARY

Degeneration. For the most part the preparation used has been the phrenic nerve with two of its roots of origin. One is crushed; the fibers of the other, remaining intact, serve as the control. Movable stimulating electrodes are applied to the nerve proper and fixed leads are taken from each of the roots separately. The results so obtained are expressed as ratios,—degenerated over normal $\times 100$. Completing observations have been made in which all of the fibers of the phrenic are degenerating, the lead then being taken first from one end of the nerve, then from the other.

Degeneration is complete in 4 days over lengths that have exceeded 200 mm.

At 3 days the changes in the area of the action potentials are such as to indicate that at the shortest distances of conduction (30 to 40 mm. from the lesion) only about one-fourth of the degenerating fibers still conduct to the central lead; and there is a further decrease in action potential area as the distance of conduction

increases. At this time half-maximal excitabilities and the conduction rates in the fastest fibers are normal in the proximal reaches but possibly decline slightly peripheralwards. The evidence indicates that the decline may not be real but merely the statistical result of a reduction in the total number of fibers conducting. Chronaxie also is normal.

The results are compatible with abrupt failures of conduction at random loci increasing in frequency peripheralwards, or possibly with failure proceeding centripetally. The histological evidence is reviewed that would account for the premised type of failure.

Under repetitive stimulation at high rates the only differences observed in the behavior of normal and degenerating fibers is such as to indicate that the relatively refractory period of the latter may be prolonged somewhat. Not all of the preparations exhibit this difference.

The suggestion is made that differences in the blood supply of different nerves possibly account for the differences, recorded in the literature, in the progression of degeneration whether centrifugal, as is generally maintained, or centripetal.

Regeneration. The properties of regenerating fibers, likewise, have been studied by the ratio and other methods in the phrenic nerve preparation.

The excitability ratio at its lowest, 29 to 31 days, declines with the distances from less than 5 to about 1, the normal being 100+. It becomes approximately uniform the length of the nerve at about 130 days, but continues to increase through 343 days, when it attains a value of about 45. At this time chronaxie is normal.

Conduction velocity in the earlier stages of regeneration falls off with the distance. At 31 days it ranges, depending upon the distance, from 7.4 down to less than 0.8, while the normal rate under identical conditions is linear and 42 mps. The rate becomes linear in regenerating fibers after about 190 days, but at 343 days has attained only slightly more than 60 per cent of the normal.

The results indicate that conduction rate and excitability may never return to normal.

Area ratios indicate that by 50 days less than half the central fibers have grown out to the peripheral end of the preparation (140 to 147 mm. from the lesion); that the number is approaching 100 per cent at about 100 days. The outgrowth is at the rate of over 4.83 mm. per day.

In the very early stages of regeneration conduction through the union may be more facile in the direction, regenerating to normal than contrariwise.

REFERENCES

- BENTLEY, F. H. AND W. SCHALPP. *J. Physiol.* **102**: 62, 1943.
BERRY, C. M., H. GRUNDFEST AND J. C. HINSEY. *J. Neurophysiol.* **7**: 103, 1944.
DAVIS, H. AND A. FORBES. *Physiol. Rev.* **16**: 407, 1936.
ELLENBERGER, W. AND H. BAUM. *Anatomie des Hundes*. p. 543, Berlin, 1891.
ENGELMANN, T. W. *Pflüger's Arch.* **61**: 275, 1895.
GRUNDFEST, H. *Trans. Am. Neurol. Assn.* p. 49, 1943.
GUTMANN, E. AND F. K. SANDERS. *J. Physiol.* **101**: 489, 1943.
HOLOBUT, W. S. AND B. JALOWY. *Ztschr. Zellforsch.* **25**: 541, 1936.

- MORRIS, H. Human anatomy. p. 1100, Philadelphia, 1942, 10th ed.
- PARKER, G. H. This Journal **106**: 398, 1933.
- ROSENBLUETH, A. AND E. W. DEMPSEY. This Journal **123**: 19, 1939.
- ROSENBLUETH, A. AND E. C. DEL POZO. This Journal **139**: 247, 1943.
- SCHOEPFLE, G. M. J. cell. and comp. Physiol. **24**: 99, 1944.
- TITECA, J. Arch. int. Physiol. **41**: 1, 1935.
- WEDDELL, G. AND P. GLEES. J. Anat., London **76**: 65, 1941.
- WEISS, P. Trans. Am. Neurol. Assn. p. 50, 1943.
- YOUNG, J. Z. Physiol. Rev. **22**: 318, 1942.
- Trans. Am. Neurol. Assn. p. 50, 1943.

RELATIVE POTENCY OF CERTAIN SYNTHETIC ESTROGENS WHEN ADMINISTERED ORALLY TO CHICKS

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An earlier communication from this laboratory (1940) reported on the relative potency of several natural estrogens in young female chicks. Since then, the interest has shifted to non-sterol estrogens, following the discovery by Dodds and his co-workers (1938) that certain dialkyl stilbenes possess estrogenic activity when tested on laboratory mammals. These investigators later (1939, 1944) concluded that the estrogenicity of the estrogens varies greatly and depends apparently on the nature of the aliphatic block in the basic molecule.

More recently, a large number of these compounds have been synthesized, which can be divided into two classes; a, Those in which the variable factor is the nature of the side chains attached to the α and β carbons, and b, those in which one or both phenolic rings are transformed into anisyl configurations.

Following the appearance of a paper by Lorenz (1943) on the use of diethylstilbestrol in fattening poultry, the subject has attracted widespread attention among poultry investigators (Jaap and Thayer, 1944; Thayer, Jaap and Penquite, 1945; Schnetzler, Andrews and Hauge, 1945; Sykes, Davidson and Barrett, 1945; Gutteridge, 1945, and unpublished material). The most recent paper on the subject by Jaap (1945) compares the potency of some fifteen stilbene, hexane, and hexadiene derivatives in chickens and turkeys.

The present report is the results of a series of three tests on the comparative potency of six estrogens³, conducted in 1944 and early 1945.

METHODS AND MATERIALS. As has been the practice at this laboratory in estrogen studies (1940, 1943), White Leghorn chicks from our own stock hatched in our incubators again were the experimental animals.

The chicks were removed from the incubator on the 22nd day of incubation, sexed by the vent method and the pullets transferred to electric battery brooders. The individual chicks were allotted to the various treatment groups by the method of restricted randomization, to minimize the effect of differences in initial body weight on the final results. In all tests, each treatment group was represented by two lots of chicks, the duplicate lots being located at random among the battery units.

The chicks, immediately on being placed in their respective compartments, were supplied, in the case of the experimental lots, with a commercial chick

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³ The estrogens were supplied by White Laboratories, Inc., Newark, N. J. A part of the cost of this investigation was also borne by this firm.

starter mash containing the appropriate estrogen. The estrogens were dissolved in 20 cc. of warm sesame oil before being incorporated into the mash. The feed was provided to the chicks ad libitum. The control group had only the oil added to their feed.

The estimated per chick consumption of estrogens was based on the total feed consumption of each lot during the trial. Variations in feed consumption between lots explains the variation between estimated levels of estrogen consumption.

On the 14th day of the test the chicks were gassed, individually weighed and prepared for dissection. The oviducts were dissected by a team of laboratory workers, which permitted the completion of the entire task in one day, despite the relatively large number of chicks involved in each trial. All weighing was done on a chainomatic balance to the nearest milligram.

In the first two tests, conducted some six months apart, four estrogens were compared: two stilbestrol derivatives, diethylstilbestrol (α, α' -diethyl-4,4' stilbenediol) and its dimethyl ether variant (α, α' -diethyl-4,4' dimethoxy stilbene), and two hexanes, hexestrol (p,p'-(1,2 diethylethylene) diphenol) and its dimethyl ether, (α, α' -diethyl-4,4' dimethoxy bibenzyl).

Each of the estrogens in these tests was assayed at three levels; 10, 20 and 30 mgm. per pound of feed. Thus in each test there were four estrogens at three levels, plus a control or 13 treatment groups in all. Since these were duplicated, there were 26 lots.

In the third test, the estrogens compared, in addition to diethylstilbestrol and dimethyl ether of hexestrol, were two unsaturated compounds: dienestrol (p,p'-(diethylidene-ethylene) diphenol) and its dimethyl ether derivative (α, α' -diethylidene-4,4' dimethoxy bibenzyl). In this test all estrogens were fed at one level only (30 mgm. per lb. of feed). With the four estrogens tested, plus a control, there were five treatment groups. Since these were duplicated, there were ten lots in all.

Altogether, 199 chicks were involved in the first trial, 241 in the second, and 146 in the third.

RESULTS. *Tests 1 and 2.* Because of the relatively small number of chicks involved in each case and because of the rather extreme variability in the oviduct weights of chicks receiving same treatment, for the purpose of statistical analysis, the duplicate lots were combined into one.

It will be noted from table 1 that the range of variation between the duplicate lots of a treatment group is considerable. Moreover, the overall results of the second test showed a much more pronounced degree of response of oviducts to estrogenic treatment. This increased sensitivity paralleled the ten gram rise in the average body weight of chicks in the second test. Undoubtedly this reflected the more optimum environmental conditions prevailing at the time of the second test, conducive to increased body growth rate and, indirectly, to a lowered threshold of oviducal response to extraneous estrogens.

The relative standing of the four estrogens remained the same in both tests. Diethylstilbestrol (D) produced the lowest degree of response, followed, in

TABLE 1

Results of the first two tests showing increases in oviduct size as a result of administering four estrogens at three dose levels

Final body weights and coefficients of variability are also shown

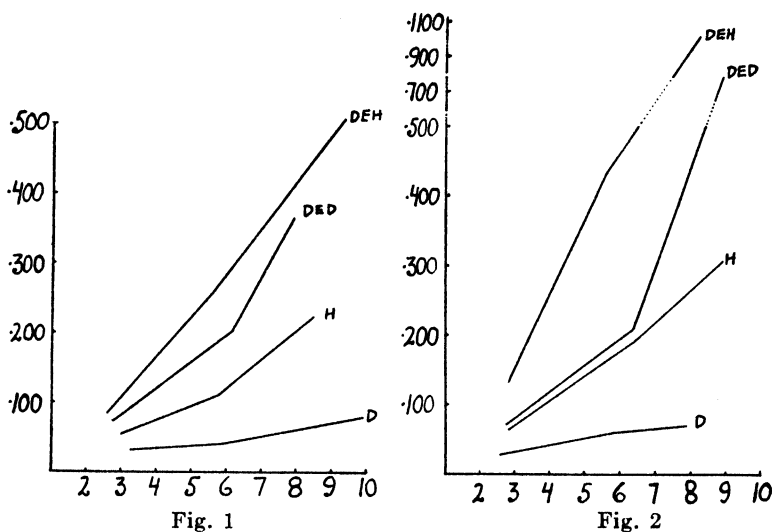
TREATMENT GROUP	TEST 1					TEST 2				
	Final body wt.	Total feed consumption per chick	Estimated estrogens dose per chick	Oviduct wt., % body wt.	C.V.	Final body wt.	Total feed consumption per chick	Estimated estrogens dose per chick	Oviduct wt., % body wt.	C.V.
Control	65.1 (14)*	129.0 gms.		0.020	30.7	76.8 (18)	119.0 gms.		0.023	72.3
Diethylstilbestrol, 10 mgm./1 lb. feed	65.0 (16)	149.1	3.29	0.027	45.4	77.4 (20)	118.8	2.62	0.027	28.3
Dimethyl ether of diethylstilbestrol 10 mgm./1 lb. feed	72.7 (16)	132.0	2.91	0.074	62.7	83.8 (20)	125.9	2.78	0.071	93.6
Hexestrol, 10 mgm./1 lb. feed	66.6 (14)	141.4	3.12	0.056	55.3	73.7 (16)	128.9	2.85	0.065	58.9
Dimethyl ether of hexestrol 10 mgm./1 lb. feed	65.0 (14)	125.9	2.78	0.083	69.6	72.1 (22)	128.6	2.84	0.134	82.0
Diethylstilbestrol 20 mgm./1 lb. feed	63.8 (16)	135.4	5.98	0.044	47.1	79.7 (20)	131.4	5.80	0.059	34.7
Dimethyl ether of diethylstilbestrol 20 mgm./1 lb. feed	66.6 (17)	142.0	6.28	0.208	149.7	79.0 (15)	141.2	6.24	0.209	66.6
Hexestrol, 20 mgm./1 lb. feed	64.4 (16)	128.6	5.68	0.123	30.6	76.4 (17)	145.5	6.42	0.195	104.6
Dimethyl ether of hexestrol 20 mgm./1 lb. feed	63.7 (12)	127.1	5.62	0.251	107.9	77.4 (19)	126.4	5.58	0.432	52.6
Diethylstilbestrol, 30 mgm./1 lb. feed	65.2 (19)	150.3	9.96	0.079	57.7	78.7 (15)	119.7	7.92	0.069	186.8
Dimethyl ether of diethylstilbestrol, 30 mgm./1 lb. feed	61.8 (12)	117.0	7.74	0.365	72.3	77.9 (16)	135.2	8.97	0.769	85.0
Hexestrol, 30 mgm./1 lb. feed	65.6 (15)	128.7	8.52	0.232	86.4	85.1 (22)	133.2	8.82	0.305	70.1
Dimethyl ether of hexestrol, 30 mgm./1 lb. feed	59.2 (18)	140.6	9.30	0.517	108.5	78.0 (21)	127.3	8.43	0.982	49.4

* Number of chicks on killing day.

order of increased estrogenicity, by hexestrol (H), dimethyl ether of diethylstilbestrol (DED) and dimethyl ether of hexestrol (DEH). This order remained unaltered at all dose levels, although there was an indication that DED at the 30 mgm. level showed a greater relative gain in potency than did DEH (i.e., relative to their potency at lower dose levels). This was true in both the first and the second test.

The relative position of the four estrogens can be visualized better by referring to figures 1 and 2.

The agreement between the two tests in the shape and relative position of the respective curves is striking, pointing to a conclusion that the indicated



Figs. 1 and 2. Relative potency of four estrogens: diethylstilbestrol (D), dimethyl ether of diethylstilbestrol (DED), hexestrol (H), and dimethyl ether of hexestrol (DEH). Abscissa: estimated total estrogen dose; ordinate: oviduct weight as per cent of body weight. Fig. 1—first test; fig. 2—second test.

rating was not spurious. In spite of the extremely high coefficients of variability (table 1), it undoubtedly represents the true relative standing of the four estrogens.

Test 3. As already mentioned in this series, two new compounds, dienestrol (Di) and its dimethyl ether derivative (DEDi) were compared in addition to diethylstilbestrol and dimethyl ether of hexestrol.

The new compounds are characterized, structurally, by two double bonds by which $\text{CH}=\text{CH}_2$ aliphatic radicles are attached to the α and β carbons. In one of these compounds (DEDi), furthermore, the anisyl configuration is substituted for the original phenyl ring structure.

The number of chicks used in each of the ten lots, representing five treatment groups, was about twice that used in the comparable units of the first two trials. However, the estrogens were assayed only at the 30 mgm. level. In every other

way, the third trial had followed the procedure adopted in the previous two tests. Results of the test are summarized in table 2.

It will be seen that the fully saturated hexane compound, DEH, again produced the greatest degree of oviduct stimulation, followed by Di the dehydrogenated derivative of D, then by DEDi and, finally, by D. Fitting these results into the data in table 1, it seems clear that dianisylhexadiene is less potent than hexestrol. The increasing order of potency of the six estrogens within the dose levels tested is, therefore, as follows: Diethylstilbestrol, dianisylhexadiene (dimethyl ether of dienestrol), dienestrol, hexestrol, dianisylhexene

TABLE 2

Results of third test showing how dienestrol and its dimethyl ether compare with the least active (diethylstilbestrol) and most active (dimethyl ether of hexestrol) compounds of the previous tests

All compounds fed at 30 mgm./1 lb. feed

TREATMENT GROUP	FINAL BODY WEIGHT	TOTAL FEED CON-SUMPTION PER CHICK	ESTIMATED ESTRO-GEN PER CHICK	OVIDUCT WT., % BODY WT.
		gram.	mgm.	
Control	84.7 (29)	134.2		0.024
Diethylstilbestrol	84.7 (30)	135.8	8.99	0.070
Dimethyl ether of di- enestrol	86.6 (28)	146.7	9.72	0.104
Dienestrol	81.4 (30)	129.7	8.59	0.208
Dimethyl ether of hexestrol	76.5 (29)	133.4	8.83	0.649

(dimethyl ether of diethylstilbestrol), dianisylhexane (dimethyl ether of hexestrol).

DISCUSSION. One of the salient facts emerging from this series of tests is the extreme variability in the response of individual oviducts to estrogen treatment. This emphasizes the danger of drawing conclusions concerning the relative estrogenicity of these compounds in chicks from isolated tests based on small numbers.

In the present study, an attempt was made to meet statistical requirements for a critical evaluation of the data of the nature dealt with in these trials (1) by using replicated units and (2) by repeating twice one of the two series of comparisons. That the attempt was at least partially successful can be seen from the already reviewed data dealing with the first two trials and also by referring to table 3. This table is based on the method of determining the numbers of individuals required at 19:1 odds to provide statistically sound comparisons between treatment groups. The method outlined by Bird and

Gutteridge (1934) is based on the degree of variability within the two populations compared.

TABLE 3

Showing required numbers to make difference reach level of statistical significance (odds of 19:1) and actual numbers employed in each case

DED ₁₀	6*											
	32†											
H ₁₀	10	42										
	30	30										
DEH ₁₀	8		48									
	30	30	28									
D ₂₀	16	16	58	18								
	32	32	30	30								
DED ₂₀	16	48	32	46	1,526							
	33	33	31	31	33							
H ₂₀	2	18	8	4	4	96						
	32	32	30	30	32	33						
DEH ₂₀	12	22	16	22	14	1,194	36					
	28	28	26	26	28	29	28					
D ₂₀	10	2,004	32	3,458	22	44	40	20				
	35	35	31	33	35	36	35	31				
DED ₂₀	4	6	6	8	52	70	10	100	8			
	28	28	26	26	28	29	28	24	31			
H ₁₀	8	20	10	14	8	3,436	26	58	14	6		
	31	31	29	29	31	32	31	31	34	27		
DEH ₃₀	10	14	12	1,338	10	414	18	50	14	160	40	
	34	34	32	32	34	35	34	30	37	30	33	
C	36	4	6	6	6	20	2	10	8	44	6	10
	30	30	28	28	30	31	30	26	33	26	29	32
	D ₁₀	DED ₁₀	H ₁₀	DEH ₁₀	D ₂₀	DED ₂₀	H ₂₀	DEH ₂₀	D ₂₀	DED ₂₀	H ₂₀	DEH ₂₀

* Required numbers to make the test valid.

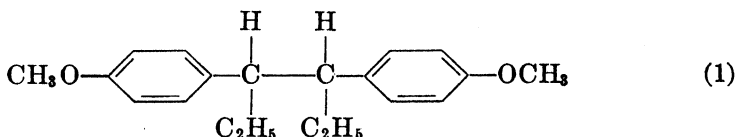
† Actual numbers.

The results tabulated in table 3, as could be expected, indicate, in conjunction with table 1, that the required numbers depend both on the variability within the two populations compared and on the extent of the quantitative difference between the two.

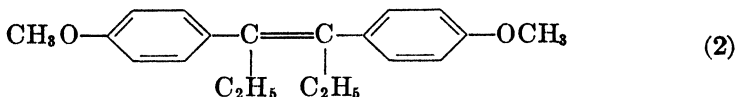
As far as the work reported herein is concerned, it is clear that in 28 cases the numbers involved were not sufficiently great to meet the requirements of the statistical test referred to above, even though the general tendency was unmistakable when presented in graphical form (figs. 1 and 2). As the main object of the present study was primarily to establish the relative potency of these estrogens, that fact does not detract greatly from the validity of the test. On the other hand the importance of having sufficient numbers becomes clear in planning quantitative assays.

Bearing the above points and reservations in mind, results of the three trials suggest that in chickens the potency of the six estrogens tested, all other things being equal, depends (1) on the degree of saturation of the α and β carbon atoms, and (2) on the nature of the peripherally attached radicles.

The two most active compounds tested were dimethyl ether of hexestrol,

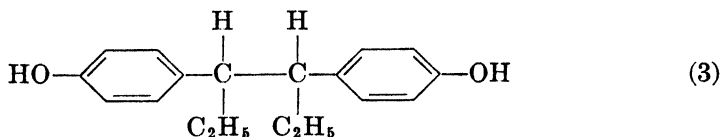


and dimethyl ether of diethylstilbestrol.

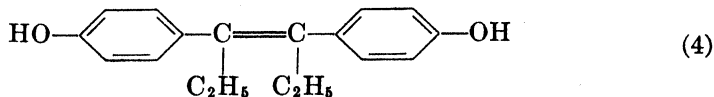


The fact that the first mentioned, a saturated meth-oxo compound has displayed greater activity than the unsaturated variant (2), is at least suggestive. The theoretically greater chemical stability of the former is paralleled by its greater physiological potency.

Again, a dihydroxy form of the *n*-hexane, hexestrol,



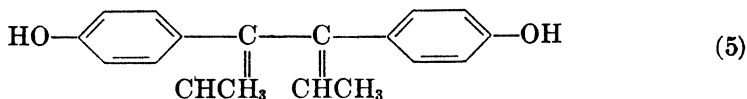
is estrogenically more potent than the unsaturated diethylstilbestrol



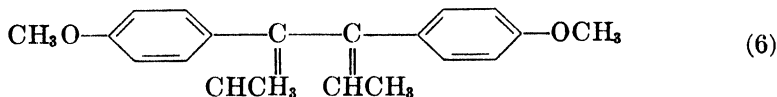
This lends weight to the above hypothesis.

On the other hand, the methylation of phenolic rings, in the para position, with the resultant anisyl configuration, such as in DEH and DED (compare 1 and 2 with 3 and 4) greatly augments the estrogenicity of the basic molecule.

The dehydrogenation of the ethyl group attached to the α and β carbons, however, results in lowered estrogenic potency. Thus both, dienestrol



and its dimethyl ether variant,



were lower in estrogenic potency than H, DED and DEH.

The above results agree, on the whole, with the rating of synthetic estrogens recently reported by Jaap (1945). However, there are certain differences of which at least one is worth considering. It concerns the relative potency of DEH and DED. At Ottawa, the former, in both tests (figs. 1 and 2), was shown to be the most potent estrogen of the six tested; Jaap on the other hand reported that when used in dosages of more than 20 mgm. per pound of feed, DED was the more potent one of the two. This difference is interesting but difficult to explain on the basis of known facts. It is possible, of course, as was illustrated in the first of the three tests reported here, that the sub-optimal growth rate of chicks apparently prevents full expression of the oviduct stimulating potency of the estrogen. It will be recalled that in the first test, the meth-oxy hexane (DEH) at the 30 mgm. level showed a smaller relative gain in potency than DED did. A possible explanation is that under such sub-optimal conditions the asymptote of the curve of estrogenically stimulated organ growth is reached sooner than when the conditions are at their optimum.

The second test, although providing such optimum conditions, nevertheless exhibited the early symptoms of this phenomenon: The DED and DEH graphs crossed when projected beyond the levels studied.

It is not likely that breed differences are responsible for the discrepancy in the results secured at the two locations as there is no known evidence to suggest the existence of estrogen dose level specificity in animals.

CONCLUSIONS AND SUMMARY

The relative estrogenic potency of the six synthetic estrogens, compared on the basis of oviduct stimulation in young White Leghorn chicks, varies with the degree of saturation in the aliphatic block of the molecule, and the nature of side chains attached to 4-4' and α - β positions. Apparently estrogenicity of these compounds increases with increasing saturation in the aliphatic block of the molecule and with methylation of the side chains.

The descending order of potency of the six compounds within the dose range employed is as follows: Dianisylhexane (dimethyl ether of hexestrol), dianisyl-

hexene (dimethyl ether of diethylstilbestrol), hexestrol, dienestrol, dianisylhexadiene (dimethyl ether of dienestrol), diethylstilbestrol.

There is some evidence that at dose levels above 30 mgm. per pound of feed dianisylhexene may prove more potent than dianisylhexane. This point requires further investigation.

The degree of variability in oviduct response rises with the increased dose level of estrogens. This fact suggests that for assay purposes, the dosages should be kept as low as possible to reduce the extent of this variability to a minimum. Moreover, it is important to have the numbers great enough in each group to overcome this handicap of high variability.

The growth rate of the chicks, reflecting their physiological well-being, greatly affects the degree of oviduct response.

Because of the inherent variability in the character studied (oviduct stimulation), the principle of replication, repetition and unity of time should be adhered to in comparing the potency of estrogens by the chick oviduct method.

REFERENCES

- BIRD, S. AND H. S. GUTTERIDGE. *Sci. Agric.* **14**: 10, 1934.
DODDS, E. C., L. GOLBERG, E. I. GRUNFELD, W. LAWSON, C. M. SAFFER, JR. AND R. ROBINSON. *Proc. Roy. Soc. London Ser. B.* **132**: 83, 1944.
DODDS, E. C., L. GOLBERG, W. LAWSON AND R. ROBINSON. *Nature* **141**: 247, 1938. *Proc. Roy. Soc. London Ser. B.* **127**: 140, 1939.
GUTTERIDGE, H. S. *Proc. Ontario Poul. Conf.*, pp. 35-38, 1945.
JAAP, R. G. *Endocrinology* **37**: 369, 1945.
JAAP, R. G. AND R. H. THAYER. *Poul. Sci.* **23**: 249, 1944.
LORENZ, F. W. *Poul. Sci.* **22**: 190, 1943.
MUNRO, S. S. AND I. L. KOSIN. *Endocrinology* **27**: 687, 1940. *Poul. Sci.* **22**: 330, 1943.
SCHNETZLER, E. E., F. N. ANDREWS AND S. M. HAUGE. *U. S. Egg and Poultry* **51**: 554, 1945.
THAYER, R. H., R. G. JAAP AND R. PENQUITE. *Poul. Sci.* **24**: 483, 1945.

STANDARDIZED HEMORRHAGIC SHOCK IN THE GUINEA PIG

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The removal of a large quantity of blood leads to a state of severe hypotension; upon reinfusion of the blood lost an effective circulation is re-established; recovery may be permanent or it may only be temporary so that in a few hours the blood pressure again falls to a low level and death supervenes, due to the development of hemorrhagic shock which is irreversible to transfusions.

It has been demonstrated that the development of this type of circulatory failure depends as much on the magnitude of the hemorrhage as on the duration of the posthemorrhagic hypotension (7). On this basis, Huizenga et al. (2) and Wiggers et al. (9) have described a method for producing irreversible hemorrhagic shock in dogs, by a two-stage bleeding and the production of successive periods of moderate and drastic hypotension, with and without anesthesia, respectively. Most other methods described are variations of the latter, being functions of blood pressure determinations and of a time factor. As an added criterion Arimoto et al. (1) have used plasma CO₂ determinations.

Attempts to produce hemorrhagic shock by the removal of a fixed quantity of blood per unit of body weight or as a percentage of the measured blood volume have not been successful because of the wide individual variations both in the ratio of the blood volume to body weight and in the ability of the organism to compensate for blood loss. Walcott (6) has described a method based on the determination of the bleeding volume; he produces hemorrhagic shock by the removal of all the blood a dog can lose in a single rapid bleeding and the immediate reinfusion of 25 per cent of the blood collected, the whole procedure being terminated in less than 10 minutes; the duration of the survival period is inversely related to the extent of the blood volume reduction; if the whole of the blood removed is returned to the circulation there is 100 per cent recovery. Sayers et al. (4) have produced hemorrhagic shock in the rat by the removal of about 3 per cent of the body weight of blood in one hour. At the first signs of respiratory collapse the animals are transfused with an inadequate volume of blood; when a second respiratory collapse is imminent, a second transfusion is given sufficient to completely replace the blood originally withdrawn.

The method described in this paper is an attempt to get away from any arbitrarily set pattern, be it blood pressure level, amount of blood removed, a time factor, or blood chemistry determinations; in other words, the animal is left to bleed completely unhampered so that the organism with its individual variations determines both the amount of blood lost and the degree and duration of hypo-

¹ This work was begun at the Department of Physiological Chemistry, Yale University, under the direction of Dr. C. N. H. Long, while the author held a Coxe Fellowship.

tension. Using the guinea pig, it has been possible to eliminate more easily than in larger animals a number of factors which undoubtedly play a part in the production of shock, such as an unnatural supine position, immobilization, and temperature changes.

METHODS. All procedures are carried out in a thermostatically regulated room, at 28°C. (82°F.) with a relative humidity varying from 65 to 75 per cent. Male guinea pigs are used, their body weight averaging 420 grams (range 380–520); they are deprived of food for about 8 hours previous to bleeding.

Without anesthesia. Through gentle handling the animals can be secured in the supine position on an operating board with a minimum of struggling. The left carotid artery is exposed under novocaine infiltration (1 ml. of a 1 per cent solution of novocaine hydrochloride) and a glass cannula snugly fitted into the artery, tied, and sewed to the skin so that it becomes firmly attached to the animal. The cannula (2 cm. long) is attached to a soft transparent rubber tubing (2 cm. long) which can be interchangeably fitted into syringe tips or blocked by a small piece of glass rod. Heparin (0.4 ml. of a 0.2 per cent solution) is introduced into the circulation from a tuberculin syringe. The animal is then freed from the operating board, having remained in the supine position less than 10 minutes.

By means of a piece of cloth the guinea pig is suspended over the table in its natural position, the four paws being introduced into four corresponding holes and each pair held together with adhesive tape. The head is maintained parallel to the body so that the cannulated artery protrudes rigidly in front of the animal, which, however, is not immobilized, but is free to move and shake all parts of its body. A 20 ml. syringe rinsed in dilute heparin solution is fitted into the cannula and suspended in front of the animal, slightly above the heart level. The blood immediately rushes into the syringe; the bleeding is not regulated in any way. Almost at once there is a period of great excitability and the animal moves and shakes considerably, being lightly restrained only at the neck region so that the relation of the cannula to the artery remains undisturbed. More and more blood rises into the syringe; meanwhile the animal passes into a state of depression, to be followed by a state of coma, which gradually leads to acute cardio-respiratory failure if untreated. The latter is preceded by 3 constant symptoms: 1, a dulled sensorium evidenced by a lack of response to pinching of the paws; 2, dying gasps which may be numerous and last for a few minutes; 3, the disappearance of the lid reflex. As soon as the latter sets in, death is imminent and 25 per cent of the blood collected is directly reinfused from the same syringe into the artery at a rapid but intermittent rate, and the rubber tubing compressed in order to prevent any back flow into the syringe. This ends the first stage of the hemorrhagic hypotension.

Effective circulation is re-established almost at once and the three symptoms disappear. Yet this amount of blood is quite inadequate so that the latter soon reappear in the same order. A second cardio-respiratory collapse being imminent, the remaining 75 per cent of the blood collected is returned to the circulation. This ends the second stage of the hemorrhagic hypotension. The syringe is then replaced by the glass rod. The animal again becomes conscious and

within a half hour is even restless and usually empties his bladder. The survival time is determined; animals still alive at the end of the 24 hour period are taken down and returned to their cages.

With anesthesia. One group of animals was anesthetized. The evanescent barbiturate, pentothal sodium, was used; a single dose of 15-20 mgm. per kilo was injected intraperitoneally as a 1 per cent solution. The procedure followed was identical to that described for the unanesthetized group, except that the bleeding was not started until one hour after the animals were suspended in order to give time for most of the anesthesia to wear off. In spite of this, the animals did not develop the period of intense excitability characteristic of the unanesthetized group.

RESULTS. Observations have been made on 32 unanesthetized guinea pigs and 21 under light barbituric anesthesia. The events which follow the free flow of blood from a cannulated artery in heparinized animals have been compared in the two groups and the data are summarized in table 1; each figure represents

TABLE 1
Standardized hemorrhagic shock in guinea pigs

	NUMBER OF ANIMALS	BLEEDING VOLUME			HEM. HYPOTENSION (IN MIN.)			DIED OF SHOCK	SURVIVED
		ml.	% B.W.	% S.A.*	First stage	Second stage	Total		
Without anesthetic	32	20 (14-31)	4.8 (3.9-6.9)	4.3 (3.3-5.8)	24 (13-55)	16 (4-45)	40 (20-67)	30 (94%)	2 (6%)
With anesthetic	21	26 (21-33)	5.5 (4.3-6.4)	5.0 (4.0-5.6)	43 (17-85)	22 (8-60)	65 (39-102)	17 (81%)	4 (19%)

* Surface area calculated according to Meeh's Formula: $S.A. = K W^{2/3}$; where $K = 8.5$.

the average of the total number of animals, the extreme variation appearing in parentheses.²

It is obvious that there are wide individual variations; it is impossible to predict how long the animal will bleed and how much blood he will lose. And yet, if a relatively large number of animals are studied a normal curve type of distribution is obtained, where the extreme variations are few and the great majority of the values fall very close to the average figure; therefore, the averages of both groups can be compared.

In the unanesthetized animals very close to half of the bleeding volume rushes into the syringe within the first two to three minutes; during the next 5 to 10 minutes almost no blood is lost and the animal is in a state of great excitability and moves considerably; as the restlessness subsides the bleeding is resumed, until the bleeding volume is very gradually completed, the last drops usually

² Hypotension is a natural sequence of hemorrhage; this method does away with blood determinations of any kind; yet we have followed the blood pressure changes in a few guinea pigs and have obtained the hypotension curves typical of hemorrhage; this justifies the subdivision of the duration of the experiment into two stages of hemorrhagic hypotension.

accompanying the dying gasps. The whole procedure is considerably slower in anesthetized animals, the initial bleeding less intense and the period of excitability although apparent is not accompanied by the furious movements characteristic of the other group. As shown in table 1 this *first stage* lasts, on the average, 24 minutes in the first group and 43 minutes in the second group, that is 19 minutes longer in the anesthetized animals.

The *second stage*, which is the interval between the infusion of 25 per cent of the bleeding volume and the second imminent cardio-respiratory collapse, lasts an average of 16 minutes in the first group and 22 in the second group, that is 6 minutes longer in the anesthetized animals. The average *total* duration of the hemorrhagic hypotension is therefore 40 minutes without anesthesia and 65 minutes with anesthesia; this difference is significant.

Similarly there is a significant difference in *bleeding volume*; it averages 4.8 per cent of the body weight in the unanesthetized animals and 5.5 per cent in the other group; the difference is of the same magnitude when calculated as percentage of the surface area.

Of the unanesthetized animals, 94 per cent died (2 survived) while of the anesthetized animals 81 per cent died (4 survived). Of the latter group all animals died within the night, that is, from 12 to 20 hours after the transfusion. In the first group, on the other hand, only 10 animals lived until the evening, the other 2 died in an average of 4 hours (range 1 to 8) after the transfusion.

Consequently it can be concluded that although the anesthetized animals lose more blood and the period of hypotension is more prolonged, the state of shock is less severe than in the unanesthetized animals since the mortality is slightly lower and the survival time considerably longer. Individually, no relation whatsoever can be established between survival time, duration or degree of hemorrhage.

At autopsy intestinal changes characteristic of hemorrhagic shock were present in the same intensity in both groups. They were limited to the small intestine which showed intense congestion of the mucosa and hemorrhagic fluid in the lumen; in a few animals similar changes occurred in the duodenum and gastric mucosa. The spleen was always contracted. At gross examination, the adrenals appeared normal. The lungs were normal.

DISCUSSION. It has been repeatedly emphasized that the development of shock is affected by a number of elements subject to considerable variation and which, not being directly related to the method used for its production, are often disregarded. One of the most important of these is the environmental temperature; wide variations in survival time and even in mortality rate between experiments performed in summer and in winter are a common occurrence, even more so when dealing with small animals. In order to eliminate this factor, all procedures have been carried out in a thermostatically regulated room. The rather elevated temperature of 28°C. (82°F.) has been chosen in order to minimize the survival time since shock may be produced more readily if the animals are kept in a warm surrounding (8).

Another important factor, rather difficult to eliminate in large animals, is the

immobilization associated with an unnatural supine position. By this method it has been possible to maintain the guinea pigs in their natural horizontal position by suspending them in a piece of cloth; nothing is tightly adjusted, movements are not restrained, there are no painful stimuli since the neck incision is infiltrated with novocaine, and consequently there is no struggling at any time. The 6 animals which were alive at the end of the 24 hour period were taken down, the arterial cannula was removed, and they were returned to their cages; none died subsequently and there was no evidence of infection even though no aseptic precautions were taken.

Anesthesia is another most important factor, recognized as affecting the course of shock in an appreciable way. It became possible to standardize this method in unanesthetized guinea pigs, yet this was achieved only subsequently to its standardization in barbiturized animals. The latter group is included for comparison purposes, demonstrating that even though a small dose of the most evanescent barbiturate is used and time allowed for most of its effects to wear off before the bleeding is started, there is an evident difference. On the average, the anesthetized animal bleeds more slowly and loses more blood, and yet survives longer; apparently the shock is not as severe. A similar difference in survival time between anesthetized and unanesthetized dogs has been reported by Wiggers et al. (9). The clinical picture differed only in the response to the first few minutes of blood loss. In the unanesthetized animals close to half of the bleeding volume rushed into the syringe within the first few minutes, bringing about a period of great excitability, undoubtedly accompanied by severe hypotension since the flow of blood is temporarily detained. In the barbiturized animals the period of restlessness although appreciable is minimal and the blood flow less abrupt at first and more gradual throughout. The slower initial loss of blood may explain the longer duration on the hemorrhagic hypotension since in the occasional unanesthetized animal which lost less than 2 per cent body weight of blood in the first few minutes, the duration of the hemorrhage was similarly prolonged. That a comparably large, rapid, initial hemorrhage is necessary to bring about the compensatory mechanism in the dog has been observed by Werle et al. (7). In both groups of animals, peripheral vasoconstriction, as evidenced by the skin changes of the nose and paws, sets in as the phase of restlessness is replaced by a phase of depression, and persists until blood is returned to the circulation.

Werle et al. (7) have emphasized that in order to produce hemorrhagic shock, the degree and duration of the post-hemorrhagic hypotension must be so intense that the failure of the heart and respiration must be threatened before starting the transfusion. At this point the balance between life and death is so delicate that the animals must be very closely watched. In the guinea pig, the disappearance of the lid reflex is almost the ultimate sign of life, and it is preceded, with very few exceptions, by a number of dying gasps. The last few drops of blood accompany the dying gasps and are probably due to them, since Woodbury and Abreu (10) have shown that they bring about a temporary rise of the arterial pressure.

The animal's own blood is returned to the circulation, to the last drop, since the relation between syringe, cannula and artery is never disturbed; the transfusion is carried out via the bleeding route, that is, intra-arterially, at a rapid but intermittent rate, in order to prevent overloading of the circulation. The advantages of the intra-arterial transfusion have been discussed by Kohlstaedt and Page (3). In order to prolong the duration of the hemorrhagic hypotension the blood is returned in two stages, as $\frac{1}{4}$ and $\frac{3}{4}$ of the amount collected, respectively; if the whole of the blood is returned at the initial collapse, most of the animals recover.

In spite of careful watch a number of animals succumbed of acute cardio-respiratory failure upon blood reinfusion. These usually died a few minutes after the transfusion, although some lived up to 30 minutes. Autopsy invariably revealed intense pulmonary hyperemia and cardiac dilatation: the typical picture of right heart failure. These animals were discarded; no confusion with those dying of hemorrhagic shock is possible, not only because of the short survival time, but also because of the autopsy findings, the lungs being normal after death from hemorrhagic shock. It is interesting to note that a greater number of barbiturized animals died of cardio-respiratory failure (23 out of a total of 44 as compared to 8 out of 39); such difference is difficult to explain as mere coincidence.

The advantage of this method resides in the fact that each animal loses a maximum amount of blood, as determined only by the organism's compensatory mechanisms, while the duration of the hemorrhagic hypotension is regulated only by the imminence of death. Blood pressure determinations as well as all other factors so often criticized in their use as criterion of the development of shock, have been eliminated. The clinical picture seen in these animals as they develop hemorrhagic shock is entirely compatible with the observations made on the severely wounded in forward field hospitals, as described by Stewart and Warner (5). We quote: "It was clear that no single factor could be used in setting up a criterion for gauging the severity of shock or the efficacy of restorative measures. Perhaps the most constant feature of the compensatory reactions in severe wound shock was the delicacy of the balance achieved and the rapidity with which a precarious adjustment could be improved or made worse."

SUMMARY

1. A method is described for the standardization of hemorrhagic shock in the unanesthetized guinea pig as well as in barbiturized animals. The results are compared.

2. The method consists of letting the heparinized animal lose blood from a cannulated artery, the flow being completely unhampered; neither the magnitude nor the duration of the hemorrhage is regulated. Once cardio-respiratory failure is imminent, 25 per cent of the bleeding volume is returned to the circulation; at the second collapse, the remainder of the blood is transfused.

3. The mortality rate of 32 unanesthetized guinea pigs was 94 per cent, and was 81 per cent in 21 barbiturized animals. On the average, unanesthetized

animals lost 4.8 per cent of their body weight of blood in 24 minutes (first stage); the hypotension was prolonged for 16 minutes (second stage). Anesthetized animals lost more blood, at a slower rate and survived longer.

4. The importance of factors such as room temperature, position, and immobilization of the animal is stressed.

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REFERENCES

- (1) ARIMOTO, F., H. NECHELES, S. O. LEVINSON AND M. JANOTA. *This Journal* **143**: 198, 1945.
- (2) HUIZENGA, K. A., B. L. BROFMAN AND C. J. WIGGERS. *J. Pharmacol and Exper. Therap.* **78**: 139, 1943.
- (3) KOHLSTAEDT, K. G. AND I. H. PAGE. *Arch. Surg.* **47**: 178, 1943.
- (4) SAYERS, G., M. SAYERS, T.-Y. LIANG AND C. N. H. LONG. *Endocrinology* **37**: 96, 1945.
- (5) STEWART, J. D. AND F. WARNER. *Ann. Surg.* **122**: 129, 1945.
- (6) WALCOTT, W. W. *This Journal* **143**: 254, 1945.
- (7) WERLE, J. M., S. COSBY AND C. J. WIGGERS. *This Journal* **136**: 401, 1942.
- (8) WIGGERS, C. J. *Physiol. Rev.* **22**: 74, 1942.
- (9) WIGGERS, H. C., R. C. INGRAHAM AND J. DILLE. *This Journal* **143**: 126, 1945.
- (10) WOODBURY, R. A. AND B. E. ABREU. *This Journal* **142**: 721, 1945.

THE EFFECT OF ASCORBIC ACID ON HEMORRHAGIC SHOCK IN THE GUINEA PIG

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Vitamin C is reported to raise the natural resistance of guinea pigs, monkeys and man, and its requirements are greatly increased during infections, in wound healing and probably under all conditions of stress; the extensive literature on the subject has been reviewed by Perla and Marmorston (6) and by Ralli and Sherry (7). Its use in the treatment of traumatic injuries as well as in post-operative shock has been strongly advocated (3). In the laboratory Stewart et al. (9) prolonged the survival time of cats bled to 50 per cent of their original blood volume by the intravenous administration of 1 gram of ascorbic acid. Locke et al. (4) observed that in rabbits 300 mgm. had a moderate beneficial effect in gravity shock. Ungar (10) greatly reduced the mortality rate from traumatic shock in guinea pigs, rats and mice, the minimal effective dose being 100 mgm. per kilo; according to this author the effect was not due to vitamin C since the oxidized preparation gave the same results. Sayers et al. (8) observed no beneficial effects with 20 mgm. of ascorbic acid administered to rats thrown into severe shock by hemorrhage.

Since all laboratory animals except the guinea pig are capable of synthesizing vitamin C, the results of studies of the effect of an excess of this substance can only be controlled and interpreted in the one exception, the guinea pig. A method for producing standardized hemorrhagic shock in this animal has been described (5) and the effect of ascorbic acid on the development of this kind of circulatory failure is reported in this paper.

METHOD. Male guinea pigs were used; their body weight averaged 397 grams (range 330–455) in the treated animals and 402 grams (range 325–570) in the controls. They were fed exclusively on fresh greens and fasted 8 hours previous to bleeding.

The procedure followed was exactly that described for unanesthetized guinea pigs in a previous paper (5). In a few words, it consists of letting the heparinized animal suspended in its natural position lose blood from a cannulated artery; the flow is completely unhampered and neither the magnitude nor the duration of the hemorrhage is regulated. Once cardio-respiratory failure is imminent 25 per cent of the bleeding volume is returned to the circulation; at the second collapse, the remainder of the blood is transfused. The environmental temperature is maintained at 28°C. (82°F.). The mortality rate as well as survival time are observed.

Ascorbic acid¹ was administered in considerably large doses: 200 mgm. (2 ml.)

¹ Vicetrin of Parke, Davis & Co. was kindly supplied by the Buenos Aires branch through Mr. J. Champalanne.

were injected intraperitoneally 15 minutes before the start of bleeding, except in 5 animals which received 100 mgm. (1 ml.) intra-arterially, via the cannulated carotid artery, 5 minutes previous to bleeding. The controls were treated in the same way, the vitamin being replaced by physiological saline.

RESULTS. The results are summarized in table 1; each figure represents the average of the total number of animals, the extreme variations appearing in parentheses.

All but 2 animals died out of 17 controls, while all but one lived out of 18 primed with vitamin C; in other words there is a mortality rate of 94 per cent in the controls, and 6 per cent in the treated animals. The only pretreated animal that died had a survival time of 20 hours; the survival time of the controls averaged 4 hours (range 1-8) in 9 animals, while 6 died during the night (from 12 to 20 hrs.). Evidently the priming of guinea pigs with ascorbic acid prevents the development of hemorrhagic shock.

TABLE 1
Hemorrhagic shock in guinea pigs

	NUMBER OF ANIMALS	DIED OF SHOCK	SURVIVED	HEM. HYPOTENSION (IN MIN.)			BLEEDING VOLUME		
				First stage	Second stage	Total	ml.	% B.W.	% S.A.
Primed with Vit. C	18	1 (6%)	17 (94%)	24 (10-70)	14 (5-28)	38 (22-98)	19 (15-28)	5.0 (4.2-7.1)	4.3 (3.5-6.2)
Controls	17	15 (90%)	2 (10%)	24 (13-38)	15 (4-45)	39 (22-66)	20 (14-26)	4.8 (4.0-5.8)	4.3 (3.3-5.2)

* Surface area calculated according to Meeh's formula: $S.A. = K W^{2/3}$; where $K = 8.5$.

It is interesting to note that both groups lost the same amount of blood; the slight difference in bleeding volume as calculated on the basis of body weight disappears when considered as percentage of body surface. Similarly, the duration of hemorrhagic hypotension was the same. In other words, the *first stage* which is the interval between the onset of the bleeding and the first imminent cardio-respiratory failure, lasts an average of 24 minutes in both groups, while the *second stage* which is the interval between the first blood reinfusion and the second imminent cardio-respiratory failure lasts an average of 14 and 15 minutes respectively, so that the *total hemorrhagic hypotension* lasts 38 and 39 minutes respectively; obviously there is no significant difference between the 2 groups. These results confirm those obtained in the unanesthetized group in the standardization of the method (5) where detailed explanations can be found.

In spite of the relatively large doses used, toxic effects attributable to ascorbic acid were not observed. There was no difference between the animals which received 100 mgm. intraarterially and those receiving 200 mgm. intraperitoneally, except that a larger proportion of the former had to be discarded for acute cardio-respiratory failure upon the blood reinfusion; this was the reason for the change in mode of administration.

DISCUSSION. The results obtained clearly demonstrate that guinea pigs primed with large quantities of ascorbic acid did not develop hemorrhagic shock, although they reacted to the hemorrhage exactly like the untreated controls, 90 per cent of which died of hemorrhagic shock. They lost the same amount of blood and the hemorrhagic hypotension lasted the same length of time, in fact, there was at no time any indication that would let one predict that these animals would survive; on the contrary, vomiting which according to Wiggers et al. (6) was seen only in dogs that subsequently died, although present in both groups was more frequent in the pretreated animals.

The quantity of vitamin C used was relatively large in an attempt to obtain a 24 hour saturation of the organism, to such a degree that its stores would not be depleted in spite of increased requirements which might be occasioned by the hemorrhage. Blood chemistry determinations were not carried out since in each case autogenous blood was used, and reinfused to the last drop.

It may be argued that the difference between the two groups was due to low levels of vitamin C in the controls. Great care was taken to keep the diet high in this vitamin; all animals were fed ad libitum exclusively on fresh greens, mostly consisting of alfalfa and carrots; they were only fasted during 8 hours previous to the onset of bleeding. The animals that survived the 24 hour period (including the 2 controls) and were returned to their cages, did not die subsequently.

The fact that ascorbic acid prevents the development of hemorrhagic shock is not easily explained. Sayers et al. (8) have recently demonstrated that in the rat hemorrhagic shock is accompanied by a marked depletion of the ascorbic acid content of the adrenal cortex and of the liver while its concentration in blood plasma is increased sixfold; furthermore there is evidence that the rat accelerates the synthesis of this substance in the liver. This active mobilization of ascorbic acid during hemorrhagic shock may soon deplete the vitamin stores of the guinea pig so that the increased requirements could not be met, explaining why the animal survives when primed with relatively large amounts of ascorbic acid. However, it may be considered that hemorrhagic shock with its characteristic irreversibility to transfusions has not been prevented by any remedial measures, except through viviperfusion of the liver of the dog as described by Fine et al. (2) who stress that by preventing liver damage, death is prevented. It has been demonstrated by Beyer (1) that ascorbic acid has a definite protective action against the experimental hepatic damage in the guinea pig. Could it not be, therefore, that by protecting against liver damage vitamin C protects the organism against irreversible hemorrhagic shock?

The prophylactic use of vitamin C has given beneficial results; it remains to be seen whether its therapeutic use will be as beneficial. Experiments are in progress to determine whether vitamin C can prevent death if administered after the blood lost has been totally reinfused, that is, once irreversibility to transfusion has been established.

SUMMARY

It has been possible to prevent the development of hemorrhagic shock in the guinea pig by pretreatment with 200 mgm. of ascorbic acid. All but one (94 per

cent) of the primed animals survived while all but 2 (90 per cent) of the controls died of hemorrhagic shock. The course followed by the hemorrhage, as to magnitude of bleeding volume and duration of hypotension was identical in both groups.

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REFERENCES

- (1) BEYER, K. H. Arch. Int. Med. **71**: 315, 1943.
- (2) FINE, J., H. A. FRANK AND A. M. SELIGMAN. Ann. Surg. **122**: 652, 1945.
- (3) HOLMES, H. N. Science **96**: 384, 1942.
- (4) LOCKE, A., R. B. LOCKE AND A. P. McILROY. Proc. Soc. Exper. Biol. and Med. **54**: 113, 1943.
- (5) PASQUALINI, C. DOSNE DE. This Journal **147**: 591, 1946.
- (6) PERLA, D. AND J. MARMORSTON. Arch. Pathol. **23**: 543, 683, 1937.
- (7) RALLI, E. P. AND S. SHERRY. Medicine **20**: 251, 1941.
- (8) SAYERS, G., M. SAYERS, T.-Y. LIANG AND C. N. H. LONG. Endocrinology **37**: 96, 1945.
- (9) STEWART, C. P., J. R. LEARMOUTH AND G. A. POLLOCK. Lancet **1**: 818, 1941.
- (10) UNTAR, G. Lancet **1**: 421, 1943.
- (11) WIGGERS, H. C., R. C. INGRAHAM AND J. DILLE. This Journal **143**: 126, 1945.

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THE RELATION BETWEEN ALVEOLAR CARBON DIOXIDE TENSION AND SUSCEPTIBILITY TO DECOMPRESSION SICKNESS¹

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Many previous studies have emphasized the wide variations in susceptibility of an individual to decompression sickness. A few recent investigations, however, have suggested that this variability is not altogether erratic. Repeated testing over long periods in this laboratory, for example, has shown that some subjects who are consistently susceptible for a few weeks may change so that they become consistently unsusceptible, and vice-versa (fig. 1A). For some the change is relatively abrupt, while others pass through a transition phase during which their behavior seemingly is erratic (fig. 1A, subjects 2 and 3). Daily testing for shorter periods (1) has shown that the tolerance of an individual frequently changes progressively rather than suddenly so that his symptoms become increasingly severe for several days, or may gradually subside (fig. 1B). In these tests several subjects varied rhythmically, with cycles of 5 to 8 days. There also appear to be diurnal variations in tolerance to decompression, revealed by higher incidence of symptoms among large groups of subjects in the morning than during any other part of the day or night (fig. 1C).

These progressive, cyclic, and diurnal changes suggest that susceptibility to decompression sickness is influenced by some physiological factors which vary in a similar fashion. It is, moreover, likely that any such variable factors would differ among individuals and so account for at least part of the contrast between susceptible and unsusceptible subjects.

One physiological factor which appears to be capable of such variations, and which may reasonably be expected to modify the susceptibility to decompression sickness, is the acid-base composition of the blood. Changes in the latter can be detected either by analysis of drawn blood, or by measurement of alveolar carbon dioxide tension. By these procedures it has been shown that the acid-base balance varies from individual to individual (e.g., 3, 4, 6) and

¹ The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Pennsylvania.

changes during the course of a single day (5, 6), as well as from one day to another (5). It is possible that such variations modify decompression tolerance for a number of reasons, among which are the following. (A) Acid-base changes alter the CO₂ content of the tissues, by changing the capacity of the blood for carrying this substance and by altering the minute volume of respiration. The changes in tissue CO₂ tension, if sufficiently great, should alter the rate of growth

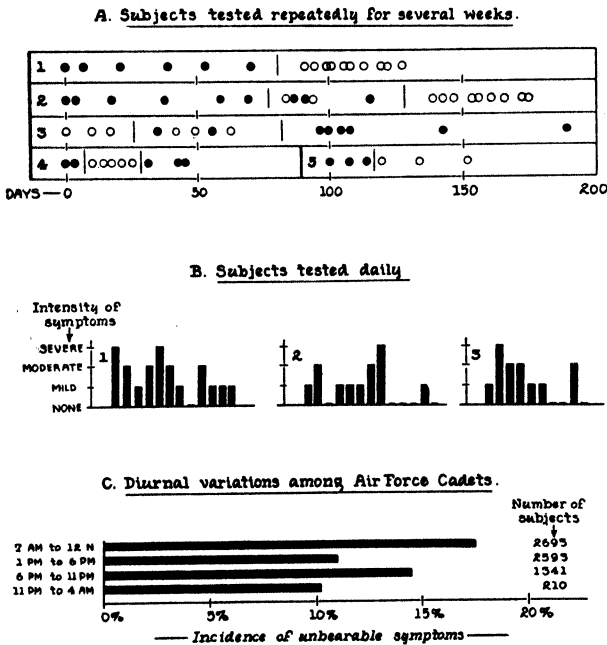


Fig. 1. Examples of variations in decompression tolerance.

A. Five subjects tested every few days for several weeks. (Two hours at a pressure equivalent to 38,000 ft.) Unfilled circles represent tests on which subjects were able to withstand decompression for the full two hours; filled circles those on which they were forced to "descend." Vertical lines indicate changes in tolerance.

B. Three subjects tested daily. Height of each block indicates the severity of symptoms during a three-hour exposure to a pressure equivalent to 35,000 feet. Data from tests by RCAF (1).

C. Diurnal variations. Length of each bar indicates the per cent of subjects experiencing unbearable symptoms during 3 hours at a pressure equivalent to 38,000 feet during a particular part of the day. Number of subjects in each group indicated at right. Data from tests by USAAF (2).

of gas bubbles in decompressed individuals, for physical reasons elaborated by Harvey et al. (7). (B) CO₂ changes alter the peripheral circulation, by direct action on the smooth muscles of blood vessels and indirectly through the cardiovascular centers. Therefore one may expect some circulatory changes, which may modify the rate of denitrogenation of the tissues during decompression. (C) Acid-base changes are known to alter the irritability of peripheral nerve (8)

and of cerebral cortex (9) and may, therefore, modify either the initiation of pain impulses by gas bubbles of given size or the number of afferent impulses that are required to evoke a painful sensation.

A relationship between carbon dioxide tension and "caisson disease" was suggested in 1896 by Snell, who was physician for workmen excavating the Blackwall Tunnel in England. During this undertaking the pressure was kept at the desired level by adjusting the number and speed of the air compressors so as to balance the rate of leakage of air from the tunnel. Since this leakage was quite variable, largely in accordance with the resistance offered by the type of soil encountered, the pumping rate and hence the ventilation of the tunnel also differed widely from day to day. Snell summarized the incidence of caisson disease as a function of the pumping rate on those days when the pressure was maintained within "a few pounds" of 20 pounds per sq. in., and revealed a striking relationship (table 1). This he explained by assuming that the incidence of caisson disease increased with the amount of expired carbon dioxide accumulating in the tunnel. This interpretation was questioned by Greenwood (1908) who was unable to produce similar effects experimentally in man or animals. Greenwood, however, does not give sufficient details to show that

TABLE 1

VENTILATION RATE (CU. FT. PER MAN PER HR.)	CAISSON DISEASE (CASES PER 100 DAYS)
Below 4,000	28.5
4,000 to 8,000	19.1
8,000 to 12,000	11.2
Above 12,000	0

his own observations were adequately controlled. It is certainly true that the correlation observed by Snell might be due to accumulation of other contaminants of the tunnel air, or to variations in the muscular exertion by the workers (16) which presumably depended on the type of soil and would, therefore, be indirectly related to the pumping rate. Moreover it may be calculated that even with ventilation as low as 4000 cu. ft. per man per hr. the average CO₂ tension could not have risen above 2 mm. Hg and it is very difficult to believe that this and lesser amounts in inspired air could have a physiological effect. It is, however, possible that greater amounts may have accumulated in certain parts of the working area. In agreement with Snell's explanation is a report of two divers who experienced the bends following dives in which CO₂ accumulated in their helmets, but were free of symptoms when the dives were repeated with more adequate ventilation (Behnke and Yarbrough). If these effects are indeed due to high CO₂ tension in the inspired air, they are in such a direction as to agree with our own observations, in which the carbon dioxide tension was varied by factors intrinsic to the individual rather than the surrounding air.

METHODS. The subjects for these experiments were healthy, male university students, 17 to 25 years of age. Simulated ascents to 38,000 feet were performed in a low pressure chamber usually twice weekly for each subject, succes-

sive exposures being separated by at least 2 days. Temperature in the chamber was maintained between 22 and 23°C. throughout the experiments.

Before decompression the subjects were required to sit quietly for at least 15 minutes in the low pressure chamber. After this preliminary rest period, the observer explained in detail the technique of obtaining samples of alveolar air and stressed the importance of avoiding unusual respiratory patterns and the need for a maximal, rapid expiratory effort. One or two alveolar gas samples were then collected from each subject at the end of inspiration. The samples were kept under positive pressure over mercury until analyzed in duplicate for CO₂ content by means of the Haldane apparatus. When two gas specimens were secured, the CO₂ values were averaged.

Because of the difficulty of obtaining reliable alveolar gas samples from untrained subjects, we have discarded for each individual the results of the first test in which a sample was collected. In addition, we have discarded the results of the second test whenever the pCO₂ turned out to be more than 1 mm. higher or lower than any of the individual's subsequent normal values. No data are included from subjects whose alveolar pCO₂ was determined less than three times. One test was rejected because the pCO₂ was extraordinarily low and an expiratory sample which happened to have been collected in the same experiment was much nearer the normal range. By these criteria we have attempted to base our conclusions on only the more reliable alveolar samples.

With the subjects breathing 100 per cent oxygen from ground level, they were decompressed to a pressure equivalent to an altitude of 38,000 feet at the rate of 3000 feet per minute. Five minutes after reaching 38,000 feet and every 10 minutes thereafter the subjects did three deep knee bends and three arm exercises in order to accelerate the onset of symptoms (16). The arm exercises consisted of lifting one oxygen cylinder cap in each hand from the floor to the shoulder, over the head, then back to the shoulder and to the floor to the count of about a second for each movement, while seated. In the first experiments the decompression lasted for 2½ hours, but when we discovered that subjects were forced to descend during the final half-hour on less than 4 per cent of the tests, the exposures were shortened to 2 hours. All the results are therefore reported on the basis of the first 2 hours of test. According to the terminology adopted, "pass" indicates that the subject was able to withstand the effects of decompression for at least two hours, while "fail" specifies his inability to do so. Tests terminated by intolerable pain from abdominal distention are not included. The results of the first decompression of each subject are also omitted, in order to minimize the effects of psychological factors.

Since some of the experiments involved premedication with NH₄Cl, placebos were often given. These consisted of 1 to 6 gelatine capsules, of different colors and sizes, which contained a few grams of glucose or NaCl. Specific instructions were always given with these placebos regarding times of ingestion, amount of water to be taken, precautions to avoid nausea or other gastro-intestinal disturbances—much the same procedure as when NH₄Cl was administered.

RESULTS. When the results of all decompression tests, excepting those in which NH₄Cl was given, are divided into groups according to the alveolar carbon dioxide tension before ascent, a striking relationship is revealed between the incidence of decompression sickness and the CO₂ tension (table 2).

The results show that the incidence of severe symptoms declines as the alveolar CO₂ tension of the subjects decreases. Descent was required on all of the seven tests for which the pCO₂ was above 43 mm. Hg, whereas all 5 tests were passed when the CO₂ was less than 38 mm. Hg. Intermediate incidences of severe symptoms were observed in the intermediate CO₂ ranges.

The statistical significance of these findings was investigated by calculating the mean² alveolar pCO₂ of all tests passed (40.03 ± 0.39), and of all tests failed (41.26 ± 0.28). Since the difference between the means is 2.53 times the standard error of the difference, the probability is only 0.011 that a difference of this magnitude is due to chance of sampling, and we therefore assume that the difference is significant.

TABLE 2

Incidence of symptoms requiring descent at different alveolar CO₂ tensions

pCO ₂ *	NUMBER OF TESTS	FORCED DESCENTS
(mm. Hg)		per cent
43.0-45.5	7	100
40.9-42.9	30	60
38.0-40.8	31	55
35.1-37.9	5	0

* The categories in table 2 were chosen by first grouping together the seven highest pCO₂ values, which were all followed by failures, and the five lowest which were all followed by passes. The remainder were then divided as nearly as possible into two groups of equal size.

We thus conclude that the incidence of severe symptoms in a group of subjects declines with alveolar CO₂ tension. Such a group of subjects is a mixture of individuals whose tolerance varies from day to day and others who are either consistently resistant or are consistently forced to descend. Therefore, it remains to be determined whether the correlation with alveolar CO₂ tension is due to (A) a relation between CO₂ tension and tolerance in the variable subjects or (B) a difference between the average CO₂ tensions of consistently resistant and consistently susceptible subjects, or (C) a combination of both.

Experimentally produced variations. In order to observe more clearly whether the susceptibility of an individual varies with changes in his alveolar carbon dioxide tension, we have experimentally lowered the CO₂ tension in a number of our susceptible subjects by administering ammonium chloride orally. By giving this substance in different amounts, and by combining the results with those of spontaneous variations in CO₂ tension, we have obtained CO₂

² Here and elsewhere in this paper the accuracy of the mean is indicated by its standard error.

values scattered throughout a fairly wide range. In the case of two subjects (K and P) who spontaneously became unsusceptible during the course of our observations (respectively no. 2 and no. 1 of fig. 1A), only the data obtained during the period in which they exhibited susceptibility to decompression sickness are included in the present section, since it was during this time that NH_4Cl was administered.

It was found that when the CO_2 tension was lowered, the susceptibility of some subjects was decreased while that of certain others appeared to be unaltered.

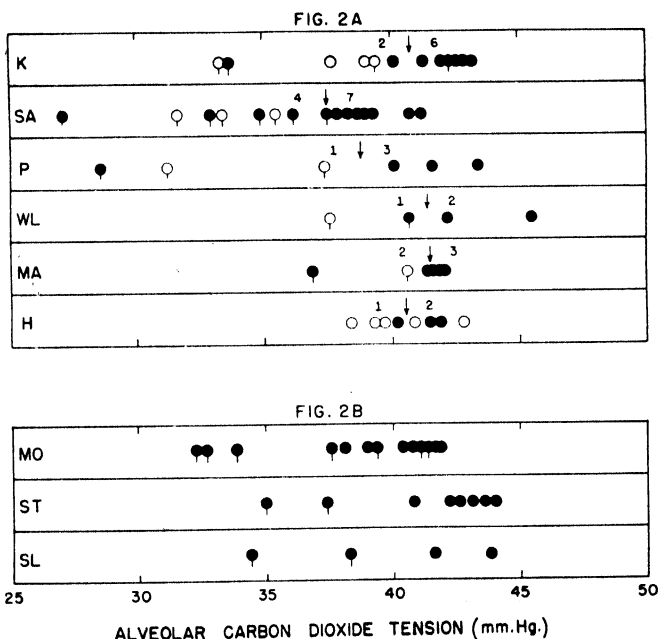


Fig. 2. Decompression tolerance as function of alveolar carbon dioxide tension. Filled circles indicate failure of two-hour test at 38,000 feet. Unfilled circles indicate passes. Short "tails" on certain circles signify that NH_4Cl was ingested before test. A. Subjects whose tolerance varied. Arrows mark median pCO_2 for each individual. Numerals beside arrows give number of tests failed in equal numbers of trials when pCO_2 was less than median (left number) and greater than median (right number). B. Subjects who failed all tests.

This is conveniently shown by arranging the results on each subject in the order of his pCO_2 values (fig. 2). It is then simple to divide the tests into two groups of equal size, one containing all CO_2 tensions greater than the subject's median value (indicated by an arrow in fig. 2A) and the other tensions less than his median. The number of failures in these two groups are shown for each variable subject by numbers beside the arrows in figure 2A.

Every one of these variable subjects had symptoms requiring descent more frequently when his CO_2 tension was high than when it was low. Indeed, except-

ing subject H, no individual passed any test when the pCO₂ was above his median. This is clear evidence that the incidence of unbearable symptoms was much reduced by a lowering of alveolar pCO₂.³

On three of our susceptible subjects we were unable to demonstrate any lowering of susceptibility by reducing CO₂ tension (fig. 2B). A plausible explanation is found by examining the location of symptoms which caused their descent. Thus in table 3A it can be seen that when their pCO₂ is lowered,

TABLE 3A

Location of symptom requiring descent in subjects who failed all tests

SUBJECT Mo			SUBJECT St		
Medication	pCO ₂	Cause of descent	Medication	pCO ₂	Cause of descent
None.....	41.5	l. upper arm	Placebo.....	43.8	l. knee
None.....	41.5	r. upper arm	None.....	43.6	l. knee
NH ₄ Cl.....	41.4	l. knee, r. leg	None.....	43.1	l. knee
NH ₄ Cl.....	41.2	r. upper arm	None.....	42.6	r. shoulder
Placebo.....	40.8	r. upper arm	None.....	42.2	l. wrist
None.....	40.4	r. upper arm	Placebo.....	40.8	l. wrist
NH ₄ Cl.....	39.4	r. heel	NH ₄ Cl.....	37.4	l. wrist
None.....	39.1	r. upper arm	NH ₄ Cl.....	35.0	l. wrist
None.....	38.1	r. upper arm	SUBJECT Sl		
NH ₄ Cl.....	37.6	r. upper arm	None.....	43.8	chokes
NH ₄ Cl.....	33.9	r. upper arm	Placebo.....	41.6	r. knee
NH ₄ Cl.....	32.7	l. upper arm	NH ₄ Cl.....	38.3	r. knee
NH ₄ Cl.....	32.3	r. upper arm	NH ₄ Cl.....	34.4	r. knee

TABLE 3B

Number of different locations in which definite symptoms were reported

SUBJECT	pCO ₂ < median	pCO ₂ > median
Mo.....	2	4
St.....	1	3
Sl.....	1	7
Total.....	4	14

each has a particular "sensitive spot" in which his symptom almost always occurred. Subject Mo, for example, descended almost always because of pain in his right upper arm. Subject St has a sensitive spot in his left wrist, and Sl

³ The significance of this relation was confirmed by calculating the mean difference between the pCO₂ observed on each test and the subject's median pCO₂. For tests passed the pCO₂ averaged 2.57 mm. Hg below the median, and for failures 0.14 mm. Hg below the median. The difference between the means was thus 2.43 mm. Hg which proved on analysis to be 2.74 times the standard error. Thus the probability was less than .006 that a difference of this magnitude was not real.

was always forced to descend by pain in the right knee, with the single exception of his test with highest CO_2 . It thus appears that these three out of nine subjects had special conditions in particular parts of their bodies which consistently caused the development of symptoms even when the alveolar CO_2 tension was considerably reduced. This is further confirmed by considering the total number of locations from which each subject reported definite symptoms, including those not severe enough to require descent. It is then found that each individual had less widely distributed discomfort when his CO_2 was low than when it was high (table 3B). This is in agreement with the idea that symptoms do not appear in the less susceptible regions when the carbon dioxide

TABLE 4
Effect of ammonium chloride

SUBJECT	AMOUNT OF NH_4Cl INGESTED BEFORE TEST							
	None		0-30 grams		30-60 grams		60-200 grams	
	No. of tests	Per cent failures	No. of tests	Per cent failures	No. of tests	Per cent failures	No. of tests	Per cent failures
1	8	100	1	100	3	67	4	50
2	13	85	4	75	4	25		
3	6	100			3	33		
4	5	100			1	100	1	0
5	6	100	1	100	2	50		
6	5	40	1	0				
7	3	100			2	100		
8	7	100			1	100	1	100
9	6	100	1	100	3	100	3	100
10	5	100			2	100		
11	5	100			1	100		

(Ammonium chloride was ingested in enteric coated tablets at the rate of 10 to 15 grams per day prior to test. Ingestion of 30 to 60 grams always lowered alveolar CO_2 below normal (average reduction = 5.7 mm. Hg), whereas some of the smaller doses did not have a significant effect on pCO_2 .)

tension is low, but that trouble persists only in certain special places where there is perhaps some local anomaly.

For our purposes ammonium chloride has largely been an experimental tool for producing greater variations in alveolar CO_2 tension than would naturally occur. Our findings, however, raise the question as to whether this substance could possibly be useful as a prophylactic agent for preventing decompression sickness. Certainly it is capable of reducing the incidence of incapacitating symptoms in some (but not all) susceptible subjects. In table 4, for example, it appears that ingestion of 30 to 60 grams of NH_4Cl at the rate of 10 to 15 grams per day increased the tolerance of the first 5 subjects, but not of subjects 7 to 11. We do not have enough data to permit an estimate of how many might be expected to benefit. There is also the obvious question of whether the medication might have side effects of an undesirable nature, although we

have had few complaints of nausea or other discomfort when the required dose was ingested in enteric-coated tablets. It is relevant in connection with the possible prophylactic use of NH₄Cl that this substance is also effective in reducing the symptoms of anoxia at lowered barometric pressure (13, 14, 15).

Spontaneous variations. Although the administration of NH₄Cl revealed a correlation between alveolar CO₂ tension and decompression tolerance, a question of greater interest is whether the variations in CO₂ tension which occur naturally are also correlated with spontaneous fluctuations in susceptibility to decompression sickness. Unfortunately only three of our subjects exhibited spontaneous variations in tolerance, but it was possible to get a large number of observations on each of them, with the results presented in figure 3A. The results of all tests completed in the absence of medication are shown for these subjects.

Each of these subjects failed more tests when his alveolar pCO₂ was above the median value than when it was below, as indicated by numbers beside the arrows in figure 3A. It can be shown statistically that the difference is more significant than these small numbers might at first suggest. The carbon dioxide tension of the tests passed averages 1.35 mm. Hg below the average for tests failed, and this difference is 2.71 times the standard error. There are thus less than 7 chances in 1000 that a difference of this magnitude is not real. It is also worth noting (fig. 3A) that no tests were failed when the pCO₂ was less than 40 mm. Hg. There can thus be no question but that tolerance increased significantly when the carbon dioxide tension fell in these three individuals.

Consistently susceptible and consistently non-susceptible individuals. We do not have sufficient data to determine with certainty whether the contrast between subjects who were never forced to descend and those who always developed unbearable symptoms is related to a difference in alveolar CO₂ tension. Our results are, however, suggestive of such a relationship.

Thus the pCO₂ of the two subjects who never failed an altitude test (fig. 3B) averaged below the commonly accepted normal value of 40 mm. Hg.⁴ The first of these (subject R, average pCO₂ 39.3 ± 0.5 mm. Hg) was clearly unsusceptible to decompression sickness as he passed all of seven control tests, plus several after various medications, and was usually completely free of even mild symptoms. The second man (G) was satisfactorily tested only four times, but he too was completely free of any definite symptoms on three of these.

Among the eight consistently susceptible subjects available for study, four always had an alveolar carbon dioxide tension above 40 mm. Hg (fig. 3C, subjects W1, St, Sl, and Ma). In view of our other findings this may have been an important reason for their low tolerance to decompression.

The next two subjects in figure 3C (Mo and Sa) differ from the preceding four in frequently having much lower CO₂ tensions. It has already been suggested that Mo is susceptible even when his pCO₂ is low because of some anomaly in his right upper arm, for it was there that his severe symptom almost always

⁴ The mean normal pCO₂ in 80 determinations during our experiments was 40.51 ± 0.23 mm. Hg.

develop decompression sickness. One category includes those whose alveolar CO₂ tension is naturally high. The other group consists of subjects whose symptoms usually recur in the same location, suggesting a local anomaly. The final two subjects in figure 3C (Wd and Sw) do not seem to fit into either of these classifications, possibly because insufficient data are available. One of them (Wd) may have had some peculiarity in his right arm, for he always (five tests) complained of intolerable pain in that extremity; but the location varied from the elbow to the shoulder, and the pain was usually rather diffuse. The remaining subject (Sw) reported severe symptoms in a different location on each of three tests.

DISCUSSION. Our findings concerning the relationship of decompression sickness to normal alveolar carbon dioxide tension may be conveniently summarized by considering three categories of subjects: 1, variable; 2, consistently tolerant, and 3, consistently susceptible. The latter can be subdivided into two groups according to their alveolar pCO₂'s.

1. Three subjects whose tolerance was variable also exhibited wide variations in alveolar pCO₂, each covering a range at least from 38 to 43 mm. Hg, with a median between 40.5 and 41.5 mm. Hg. Each failed a greater percentage of tests when his pCO₂ was high than when it was low.

2. Two consistently tolerant subjects had relatively low alveolar CO₂ tensions, the highest single value for either of them being 41.2 mm. Hg, and the highest median 39.2 mm. Hg.

3A. Four consistently susceptible subjects had relatively high alveolar CO₂ tensions, the lowest single value being 40.8 mm. Hg, and the lowest median 41.6 mm. Hg.

3B. Two consistently susceptible subjects had relatively low alveolar CO₂ tensions (down to 38 mm. Hg). Both of these men apparently had some local condition rendering a particular part of the body especially sensitive to the effects of decompression.

Two other subjects, who were perhaps not tested enough times, could not be fitted into this schema.

All of these results without medication may be still more briefly stated by saying that every subject developed severe symptoms at least occasionally when his CO₂ was above 41 mm. Hg, and that none developed severe symptoms when his CO₂ was below 40 mm. Hg, except for those who apparently had special local anomalies (and Sw who may not have been sufficiently studied). The results after NH₄Cl ingestion cannot be so simply stated, for under these conditions occasional, although significantly fewer, tests were failed even when the pCO₂ was quite low.

While the rules stated in the preceding paragraph apply to our subjects, they might well not be strictly followed in a larger survey. However, our results do indicate clearly that on the average the incidence of decompression sickness can be expected to be less when alveolar carbon dioxide tension is low than when it is high.

The reason for this relationship is unknown. Various possibilities were

suggested in the introduction, and we have made no experiments which would distinguish between them. In general the mechanisms which have been considered concern those through which changes in pH or $p\text{CO}_2$ may modify either the growth of gas bubbles or the number and effectiveness of nerve impulses initiated by such bubbles. But these speculations remain as unconfirmed, although reasonable, hypotheses.

In conclusion it is well to emphasize that susceptibility to decompression sickness is certainly related to other variable factors besides the alveolar carbon dioxide tension, for some tests are passed even when the $p\text{CO}_2$ is high, while some are failed when it is low. These other factors must differ not only from day to day and between individuals, but also between different parts of the same individual. They may be thought of as setting the critical level of alveolar CO_2 tension, above which symptoms are capable of developing in a particular location, the critical alveolar CO_2 being different for different parts of the body. No doubt these other factors are partly circulatory, influencing among other things the relation between alveolar and tissue CO_2 tensions, and partly extra-vascular phenomena, such as the amount of fat present, the effects of previous injury, and the rate of local CO_2 production.

SUMMARY

The relation between alveolar carbon dioxide tension and susceptibility to decompression sickness was investigated by comparing the carbon dioxide tension in alveolar air collected shortly before decompression with the ability of the subject to remain for two hours in an altitude chamber at a pressure equivalent to 38,000 feet.

The following results show that susceptibility increases with alveolar $p\text{CO}_2$.

1. Subjects were forced to descend whenever the alveolar $p\text{CO}_2$ was more than 43 mm. Hg (seven cases). In contrast no subject was forced to descend when his alveolar $p\text{CO}_2$ was less than 38 mm. Hg (five cases). Between 38 and 43 mm. Hg subjects were forced to descend on 57 per cent of their tests (61 cases).
2. Each subject whose tolerance varied during the course of these experiments passed fewer tests when his alveolar CO_2 tension was high than when it was low. This was true both of spontaneous variations in tolerance and changes produced by ingestion of ammonium chloride. The latter substance was effective in reducing the susceptibility to decompression sickness of some subjects, but not all.
3. The average alveolar CO_2 tension tended to be low for both of two subjects who were always resistant to decompression and high for four out of eight subjects who were always forced to descend.
4. The CO_2 tension tended to be normal or low in the four remaining consistently susceptible subjects. Two of these may have been inadequately studied, and two were characterized by the recurrence of pain in some particular region. This suggests that the latter were individuals with special local conditions which caused unusual sensitivity to decompression. A similar circumstance appeared to exist in two other individuals who always had severe symptoms even when their alveolar carbon dioxide tension was markedly lowered by ammonium chloride.

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REFERENCES

- (1) STEWART, C. D., H. S. SMITH, H. T. McALPINE, O. H. WARWICK AND G. W. MANNING. Royal Canadian Air Force, April 20, 1942. Associate Committee on Aviation Medical Research Report No. C.2178.
- (2) GUEST, M. M. Aviation Physiologist's Bulletin, United States Army Air Forces, No. 3. 9 Jan., 1944.
- (3) HALDANE, J. S. AND J. G. PRIESTLEY. J. Physiol. **32**: 225, 1905.
- (4) FITZGERALD, M. D. AND J. S. HALDANE. J. Physiol. **32**: 486, 1905.
- (5) HIGGINS, H. L. This Journal **34**: 114, 1914.
- (6) VAN SLYKE, D. D., E. STILLMAN AND G. E. CULLEN. J. Biol. Chem. **30**: 401, 1917.
- (7) HARVEY, E. N., D. K. BARNES, W. B. McELROY, A. H. WHITELEY, D. C. PEASE AND K. W. COOPER. J. Cell. and Comp. Physiol. **24**: 1, 1944.
- (8) LEHMAN, J. E. This Journal **118**: 600, 1937.
- (9) DUSSER DE BARENNE, J. G., W. S. McCULLOCH AND L. F. NIMS. J. Cell. and Comp. Physiol. **10**: 277, 1937.
- (10) SNELL, E. H. Compressed air illness. London, 1896.
- (11) GREENWOOD, M., JR. Brit. Med. J. **1**: 914, 1908.
- (12) BEHNKE, A. R. AND O. D. YARBROUGH. U. S. Nav. Med. Bull. **36**: 542, 1938.
- (13) DOUGLAS, C. G., C. R. GREENE AND F. G. KERGIN. J. Physiol. **78**: 404, 1933.
- (14) HODES, R. AND M. G. LARRABEE. Nat. Res. Council, Div. of Med. Sci., Committee on Aviation Med., Report No. 96, 1942.
- (15) BARACH, A. L., M. ECKMAN, E. GINSBURG, A. E. JOHNSON, AND R. D. BROOKES. J. Aviation Med. **17**: 123, 1946.
- (16) HENRY, F. M. This Journal **145**: 279, 1946.

THE EFFECT OF ANOXIC ANOXIA ON URINE SECRETION IN ANESTHETIZED DOGS

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Most investigators have found that anoxic anoxia causes in unanesthetized mammals, including man, an increased secretion of urine (1, 5, 11, 12, 13). Swann *et al.* (4, 18, 19) in rats exposed 24 hours to a barometric pressure of 380 mm. Hg did not find any change. On the other hand, the effect of anoxia on the anesthetized animal has been found to produce most frequently a decreased secretion of urine (2, 3, 7, 14, 20, 23), although most workers have observed occasional increases also.

In order to understand more clearly the effect of anesthesia on the renal response to anoxia and to gain a better insight into the effect of anoxia on the kidney, we have repeated and extended the experiments on the anesthetized dog. We have used degrees of anoxia from mild to severe, tried the effect of using various anesthetics and, in addition, studied the effect of various autonomic agents on the renal response to anoxia.

METHODS. The secretion of urine was followed in 30 dogs weighing 2.89 to 10.9 kgm. Sodium barbital (250 mgm./kgm.) was administered after preliminary induction of anesthesia with ether in 18 dogs and intravenously without ether in 6 others. Five dogs were anesthetized with 1 gram/kgm. urethane given intravenously. One dog was anesthetized with ether only, but further use of this agent was discontinued due to the fact that the level of anesthesia was practically impossible to control during anoxia.

Following induction of anesthesia, the ureters were exposed by a small incision through the midline of the abdominal wall and a cannula tied into each just above its entrance into the bladder. The urine was led to an automatic drop recorder¹ through a single tube connected to the cannulae by means of a Y-tube. Blood pressure was recorded by means of a mercury manometer from the carotid artery in most cases.

Anoxia was produced by administering mixtures of nitrogen and oxygen from a gas mixing machine (standardized by gas analyses) to a one-way valve system connected to a tracheal cannula. Room air was used for the control tests.

The procedure, followed as a rule, was to obtain 20 min. of a uniform control secretion, then subject the dog to anoxia for 20 min. or until respiratory or circulatory embarrassment made further continuance inadvisable. Following the anoxia, time was allowed for attainment of uniformity of secretion, then a 20 min. control period was obtained and another, or the same degree of anoxia tried, and so on. The effects of the following concentrations of oxygen in nitrogen were tested: 14, 11, 9, 7 and 5 per cent.

¹ Constructed by Dr. David F. Marsh of the Department of Pharmacology and kindly loaned for these experiments.

With 15 dogs, after the responses to various degrees of anoxia were obtained, an autonomic agent (cocaine hydrochloride, 10 mgm./kgm.; ergotamine tartrate, 0.1 mgm./kgm.; prostigmine methylsulfate, 0.05 mgm./kgm.; yohimbine hydrochloride, 1 mgm./kgm.) was injected and the responses to the same degrees of anoxia previously tried, recorded.

RESULTS. The results of 101 experiments with various degrees of anoxia in 24 dogs under sodium barbital are shown in the figure. The results with barbital

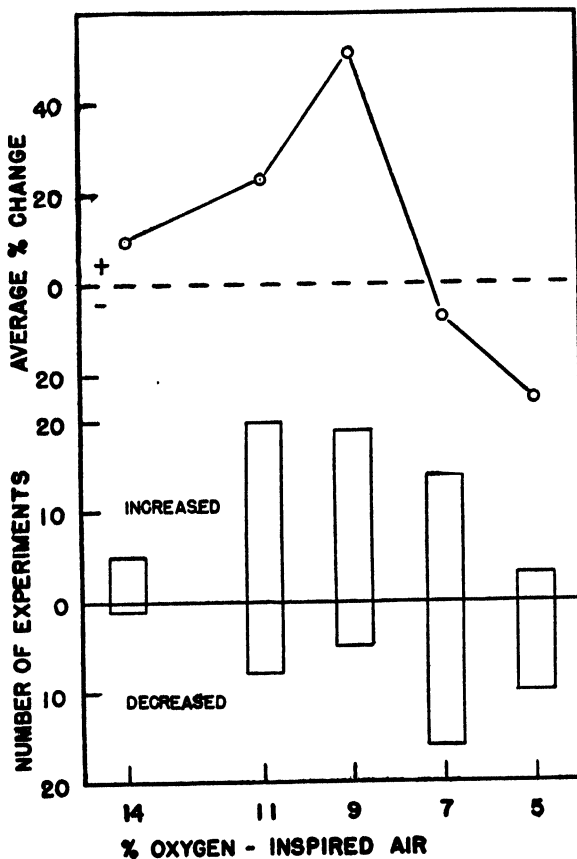


Fig. 1. Effect of anoxia on urine secretion

alone did not appear to be different from the main series, so they are included with the others. The bar graph at the bottom of the figure shows the distribution, in respect to the degree of anoxia, of the incidences of both increased and decreased urine secretion induced by the anoxia. The line graph at the top of the figure shows the average per cent change from the control rates of secretion for each degree of anoxia. The rate of secretion immediately before each exposure was used as the basis of comparison. The incidence of increased secretion passes through a maximum around 11 per cent oxygen, while that of

decreased secretion tends to increase with the severity of the anoxia. The average rate of secretion increases under the influence of oxygen want up to 9 per cent oxygen, then decreases to the control level at about 7 per cent and at 5 per cent becomes markedly depressed.

This sequence of changes, though fairly uniform when the results as a whole are considered, was rarely seen as unequivocally when the experiments on any one dog were considered. The best that can be said for the latter is that there was frequently a fairly evident trend, punctuated here and there with responses which simply appeared out of line, or were even reversals of response to the same degree of anoxia.

Of the 24 dogs used with sodium barbital, 18 developed oliguria at some degree of anoxia, usually at the more severe degrees, while in all but 2 there was a polyuria during exposure to one or more of the oxygen concentrations. Three dogs failed to show an oliguria even at 5 per cent oxygen, the lowest concentration used. Frequently the oliguria was associated with incipient failure of the circulation and/or the respiration, due to the stress of anoxia.

The types of urine secretion immediately following the period of anoxia can be classified roughly into three equal groups: (1) a return to or toward the control rate, (2) an increased rate above that of the control or (3) a reduced rate. It is noteworthy that the increased rate was an accentuation of an anoxia-induced polyuria about as often as it was an after response to an anoxia-induced oliguria. Likewise, the decreased rate was an accentuation of an anoxia-induced oliguria about as often as it was an after response to an anoxia-induced polyuria.

The results on the 5 dogs given urethane were complicated by the fact that the anesthetic produced a hemoglobinuria. The average per cent change from the control and the number of experiments performed at each grade of anoxia were respectively: +15.3 in 2 tests at 14 per cent; +35.0 in 3 at 11 per cent; +4.4 in 8 at 9 per cent, and -39.0 in 4 at 7 per cent oxygen. These results differ from those obtained under sodium barbital in that the peak of increase is at 11 per cent oxygen as though the curve of the figure had been shifted to the left.

The results obtained after the administration of the autonomic agents were disappointing. For example, after cocaine in a total of 19 experiments the response to 11 per cent oxygen was 59.7 per cent increase in rate; to 9 per cent oxygen, 6.0 per cent decrease in rate; but to 7 per cent oxygen, 19.3 per cent *increase* in rate. The results with yohimbine and prostigmine were equally inexplicable. Anoxia, after ergotamine, so reduced the blood pressure that experiments with it were given up as impractical.

DISCUSSION. These experiments confirm the results previously reported from this laboratory and of others on the effects of anoxia on urine secretion in the anesthetized dog in as much as there were both increases and decreases in rate. They show further that there is apparently a dependence of the type of response on the degree of anoxia; that is, mild anoxia as a rule produces a polyuria, while severe anoxia more generally retards or stops urine secretion.

Toth (21) found that in anesthetized dogs slow rates of epinephrine injection produce polyuria as a rule and fast rates, oliguria. As Toth pointed out, both these effects are obtained in anesthetized animals exposed to anoxia. In the present experiments there was more or less of an association of polyuria with mild anoxia and oliguria with severe anoxia. Since mild degrees of anoxia would be expected to cause less epinephrine liberation from the adrenals than severe degrees, one is tempted to attribute the changes in urine secretion in anoxia to varying rates of discharge from the adrenal medulla. But as Van Liere *et al.* (23) have pointed out, there may be a reflex mechanism operating to produce the changes as well. Toth's later experiments (22) on dogs with occluded adrenal veins and with denervated kidneys show that epinephrine secretion is not necessary for the oliguria, but that normal renal innervation is. There is further support for this view from the recent studies (6, 8, 9, 10) of renal blood flow, which have demonstrated the independence of the kidney on systemic blood pressure, particularly under various conditions of stress. Such independence has been attributed to an autonomous control of the renal vascular bed (16). Thus an anoxia-induced polyuria may be much greater than could be accounted for by the associated rise in blood pressure alone, and an oliguria may be produced by an anoxia of greater severity in spite of the more pronounced rise in blood pressure. It is probable that anesthesia in some way alters the regulating mechanism of the kidney in its response to anoxia (12) and perhaps to other forms of stress.

Since urethane is known to leave the circulation and respiration relatively unaffected, its use was tried in order to gauge the rôle of reflexes on the control of the kidney during anoxia. It will be recalled that an apparent reduction in the threshold to the urine depressing action of anoxia was found when compared to the results under barbital. The results of Schnedorf and Orr (7) and of Brassfield and Behrman (3), obtained under nembutal, indicate an even greater reduction of threshold. Thus quantitative differences in this regard among the various anesthetics seem to be present.

It was hoped that the autonomic agents in the experiments in which they were employed would upset the renal regulating mechanism in definite ways and thus elucidate the nature of the control. Unfortunately, their use has not clarified the problem appreciably, possibly because of the introduction of side effects.

Silvette (12, 15) has on the basis of studies on the response of the unanesthetized rat to anoxia held to the view that the polyuria is a result, in part at least, of failure of tubular reabsorptive function. However, in neither anesthetized nor in unanesthetized dogs exposed to anoxia did Toth (20) or Brassfield and Behrman (3) find that urinary concentrations of urea and chloride approached those of the plasma, but that the rates varied directly as the water secretion in both polyuria and oliguria. In the dog, then, no obvious evidence of tubular failure has been reported with anoxia, but increases in urine rates as high as those seen in rats have rarely been reported in dogs.

In view of the repeated occurrence of oliguria in response to anoxia in anes-

thetized animals it is difficult to explain why a similar response has not been seen in the unanesthetized animal. It is possible that time is a factor which has not been taken sufficiently into account in the latter, for Swann *et al.* (4, 18, 19) found at least no change in urine secretion after a relatively long exposure (24 hrs.) in unanesthetized rats. In the present series, one dog exposed to 9 per cent oxygen for 68 min. responded at first with polyuria, later with oliguria, but finally when circulatory and respiratory failure became imminent, with anuria. The impression gained from our experiments with anesthetics is that oliguria appears rather uniformly when the stress of anoxia approaches the limit of the homeostatic mechanisms of the animal. Yet, it has been found by one of us (J.C.S.) that unanesthetized rats exposed to progressively severe degrees of anoxia for periods of $3\frac{1}{2}$ hours have a proportionate polyuria up to the lethal level, without showing an oliguria (17).

SUMMARY AND CONCLUSIONS

In 101 experiments on 30 barbitalized dogs subjected to various degrees of anoxia (14, 11, 9, 7, and 5 per cent oxygen in nitrogen) the greatest relative incidence as well as the greatest average increase in urine secretion occurred at 9 per cent oxygen. The greatest relative incidence as well as the greatest average decrease occurred at 5 per cent oxygen. Under urethane the peak of increased secretion occurred at 11 per cent oxygen.

Experiments in barbitalized dogs exposed to anoxia before and after autonomic agents (cocaine, ergotamine, prostigmine, yohimbine) were equivocal.

It is concluded that generally mild anoxia produces a polyuria and severe anoxia, an oliguria, but the incidence of either is affected by the type of anesthetic agent used.

No adequate explanation can be given at present to account for the absence of oliguria in unanesthetized animals exposed to anoxia.

REFERENCES

- (1) ARMSTRONG, H. G. Principles and practices of aviation medicine. Williams and Wilkins Co., Baltimore, 1939, p. 284.
- (2) BINET, L., M. V. STRUMZA AND A. SAMARAS. *Compt. rend.* **209**: 576, 1939.
- (3) BRASSFIELD, E. R. AND V. G. BEHRMANN. *This Journal* **132**: 272, 1941.
- (4) COLLINGS, W. D., H. G. SWANN, C. U. DERNEHL AND J. K. KLINE. *Fed. Proc.* **2**: 7, 1943.
- (5) LANGLEY, L. L. AND R. W. CLARKE. *Yale J. Biol. and Med.* **14**: 529, 1942.
- (6) PHILLIPS, R. A., V. P. DOLE, P. B. HAMILTON, K. EMERSON, JR., R. M. ARCHIBALD AND D. D. VAN SLYKE. *This Journal* **145**: 314, 1946.
- (7) SCHNEDORF, J. G. AND T. G. ORR. *Am. J. Digest. Dis.* **8**: 356, 1941.
- (8) SCHROEDER, H. A. AND J. M. STEELE. *J. Exper. Med.* **72**: 707, 1940.
- (9) SELKURT, E. E. *This Journal* **145**: 376, 1946.
- (10) SELKURT, E. E. *This Journal* **145**: 699, 1946.
- (11) SILVETTE, H. *Proc. Soc. Exper. Biol. and Med.* **51**: 199, 1942.
- (12) SILVETTE, H. *This Journal* **140**: 374, 1943.
- (13) SILVETTE, H. *Fed. Proc.* **3**: 42, 1944.

- (14) SILVETTE, H. Ibid. **3**: 84, 1944.
- (15) SILVETTE, H. Ibid. **4**: 65, 1945.
- (16) SMITH, H. E., E. A. ROVENSTINE, W. FOLDRING, H. CHASIS AND H. A. RANGES. J. Clin. Investigation **18**: 319, 1939.
- (17) STICKNEY, J. C. To be published.
- (18) SWANN, H. G., W. D. COLLINGS, J. K. CLINE AND C. U. DERNEHL. Science **96**: 588, 1942.
- (19) SWANN, H. G. AND W. D. COLLINGS. J. Aviation Med. **14**: 114, 1943.
- (20) TOTH, L. A. This Journal **119**: 127, 1937.
- (21) TOTH, L. A. This Journal **119**: 140, 1937.
- (22) TOTH, L. A. This Journal **129**: 532, 1940.
- (23) VAN LIERE, E. J., J. S. PARKER, G. R. CRISLER AND J. E. HALL. Proc. Soc. Exper. Biol. and Med. **33**: 479, 1935.

THE MEASUREMENT OF REDUCTION TIME OF BLOOD IN THE CAPILLARIES OF THE SKIN

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One of the most fundamental factors in Cannon's (1) concept of homeostasis is the maintenance of an adequate oxygen supply to the tissues. The evaluation of this supply and its variation under changing conditions is a laborious and time-consuming process. In consequence, the tendency has been to resort to various indices such as heart rate, blood pressure changes and post-activity oxygen consumption, rather than direct observation.

The present paper deals with a direct, simple method for evaluating the supply of oxygen to the skin. In this method, as opposed to the procedure of measuring arteriovenous difference in oxygen, the measurement is a function not only of the rate of utilization of oxygen, but also simultaneously of all the factors—circulatory, metabolic and respiratory—that may alter the amount of oxygen available to the skin at any particular moment. These latter factors are of the utmost importance since the quantity of oxygen in the arterial blood is only one of many determinants of the competency of the oxygen supply to the tissues. It is far more desirable to evaluate the supply of oxygen in the capillaries, subject as it is to a variety of physiological influences, than to estimate it by inference from the saturation of the arterial blood.

Theoretical. It has long been recognized that the underlying blood contributes definitely to the color of the skin and, further, that the spectroscopic examination of the light reflected from this tissue reveals the absorption bands of oxyhemoglobin. Edwards and Duntley (2) who made extensive spectrophotometric observations of skins of various degrees of pigmentation and from different parts of the body, state that in the normal individual the characteristics of oxyhemoglobin are always present. In the course of their article, these authors point out the fact that venous compression tends to obliterate the bands of oxyhemoglobin. Following occlusion, a change in the spectral characteristics of reflected light is to be expected, since the rate of flow through the capillaries is an important factor in determining the extent to which the blood is reduced.

The nature of this change is predictable on the basis of our knowledge of the spectrophotometry of mixtures of oxygenated and reduced hemoglobin (Ray, Blair and Thomas, 3). If expressed in terms of light absorption, such a mixture would present a curve which would be the algebraic sum of the absorption factors of the two pigments (fig. 1). Even casual observation of curves *A* and *B* makes it at once evident that there exists a mixture where no discrete bands will be present, but absorption will be a rather broad plateau, extending well over the

¹ Deceased.

middle range of the spectral area under consideration. Mathematically, for solutions of the two pigments such a plateau will occur when the mixture is composed of 20 per cent oxyhemoglobin and 80 per cent reduced hemoglobin. This broad, flat plateau is detectable by the technique of spectrophotometry of the transmitted light for solutions (curve *C*). On the other hand, when observation is made with reflected light and a spectroscop of low dispersion, this difference in intensity between one part of the spectrum and another is not sufficient in magnitude to be detected and the spectrum, therefore, appears homogeneous. If one observes oxyhemoglobin under conditions where the oxygen content is being decreased by reduction, it is found that a point is reached where the bands disappear. This represents a definite and easily recognizable end point.

It is apparent, therefore, that if it is possible to control physiologic conditions

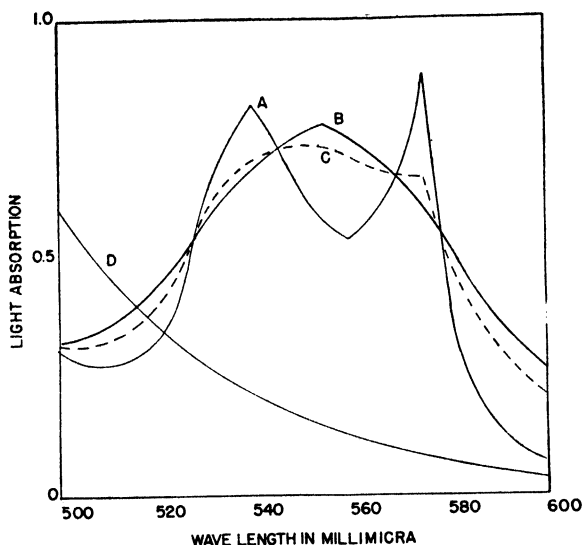


Fig. 1. Comparative absorption curves of oxyhemoglobin, *A*, reduced hemoglobin, *B*, a mixture of 80 per cent reduced hemoglobin and 20 per cent oxyhemoglobin, *C*, and melanin, *D*.

in such a way that oxygen disappears from the blood of the skin, one has a means of evaluating the rate of a standard degree of reduction of blood within the tissues under observation. This can be done simply by suddenly occluding the blood supply between the point of observation and the heart.

Previous work. The technique to be reported in this paper is the result of five or six years' observation and the correlation of the results of various physiologic studies. After a considerable period of investigation the writer was surprised to learn that a similar method had been reported in detail by Vierordt in 1878 (4) and that his observations had been extended by one of his students, Dennig (5), in 1883. Similar observations were made by Hénocque (6) (c. 1895). Meyer and Reinhold (7), in 1926, worked on rabbits and men, using a comparable technique as a measure of oxygen consumption.

Beginning with Nicolai (8), in 1932, there has been developed among German scientists a method of amplification of red and infra-red light transmitted through the tissues. Matthes (9, 10), in particular, and his co-workers have developed this method for recording changes in blood content and in oxygen saturation. These highly developed techniques are not easily adaptable to the investigation of certain phases of the problem, which may readily be studied with a technique based on a constant end point.

APPARATUS AND PROCEDURE. A diagram of the apparatus is given in figure 2. The actual dimensions may be computed from the length of the spectroscope, which is 4 inches. The frame of the apparatus consists of an angle tube, *F*, a segment of which has been removed as shown in the sketch and shaped to fit upon the skin, *S*. The skin is protected from the metal edge by a rubber ring, *R*. The construction of the tube should be such that the beam of light from the lamp, *La*, is focused upon the skin by a 33 diopter lens, *L*, and reflected toward the slit

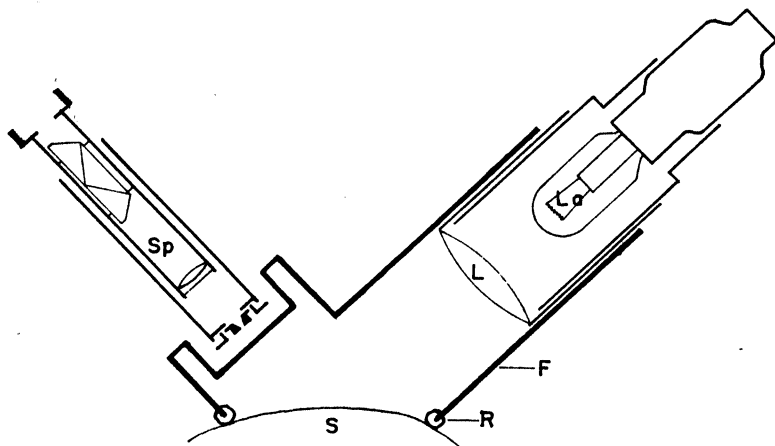


Fig. 2. Diagram of apparatus used for measuring reduction time. Description in text

of the spectroscope, *Sp*. It is not necessary to have intense illumination, but for the purpose of routine observation it is desirable that the illumination be constant. As a light source we have used an incandescent bulb designed for the Friedenwald ophthalmoscope. This is a 15 watt lamp operating at 5.75 volts. The filament is compact and sturdily mounted. It is the writer's experience that the best illumination of the skin is obtained when the relationship to the lens is such that the image of the filament appears about one foot beyond the opening of the apparatus. This gives an illuminated spot from $\frac{1}{2}$ inch to $\frac{5}{8}$ inch in diameter, which is homogeneous and of sufficient intensity to permit working with a narrow slit, thus obtaining a maximal resolution of the bands. With such an apparatus it is possible to detect the primary band of oxyhemoglobin in almost all skins, although it must be admitted that under certain conditions observation is difficult. In only three experiments in over two thousand has it been impossible to make observations, and in these cases there were clinical reasons why one should not expect to see oxyhemoglobin.

The apparatus, as it is set up for use, is shown in figure 3. The frame of the apparatus is clamped to a stand by means of a three-way adjustable bracket. The voltage reducer, required when the apparatus is used on house current, is not shown. This is sometimes fastened in an inverted position under the table. While a transformer may be used to obtain a 6 volt supply at an appropriate amperage, it has been found more convenient to use a compact series of resistances as a voltage reducer. With this arrangement, no limitation is imposed by the type of current supplied. There is no reason why a small 110 volt lamp with concentrated filament cannot be used. In this laboratory, however, it has been found more convenient to have a type of apparatus that can, when the need arises, be operated from batteries.

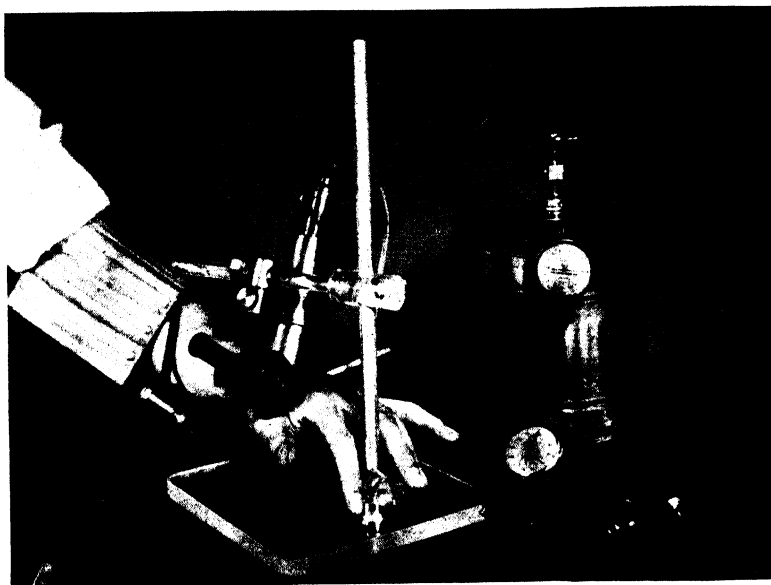


Fig. 3. Reduction time apparatus assembled for the test. Description in text

The technique of producing a sudden occlusion is carried out through the use of an ordinary sphygmomanometer cuff which is connected through a stop-cock to a 3500 cc. air reservoir. As shown in figure 3, an aneroid manometer and a pressure bulb are also in communication with the reservoir. An air chamber of this size at 250 to 260 mm. Hg pressure will inflate a properly fitted cuff to a pressure of 200 mm. Hg. It is a simple matter, therefore, to open the cock and snap a stop watch simultaneously, thereby establishing a definite starting point for timing the reaction. There is little difficulty in determining the point at which the band in the yellow region, i.e., 577 millimicra, disappears.

Direct visual observation is not especially fatiguing; over fifty subjects with at least four readings on each have been studied in a day without eye strain or other signs of fatigue. After brief experience with the technique, a site can be selected where satisfactory readings may be obtained. The selection of this site is impor-

tant, since readings must be made on blood in the smallest vessels. If, inadvertently, one includes a vein in the field, readings are extremely long; in fact, in many cases the bands of oxyhemoglobin do not disappear. It may be of advantage to allow the hand to hang down and to note the location of the large superficial vessels before selecting a spot for observation. Apparently, it is not the venous saturation which determines the end point, but the saturation of the capillary blood following the attainment of a point of stasis.

The activity of the capillaries themselves should not be disregarded. Resting capillary beds undergo step-like increases following occlusion of the arterial supply. This is obvious when one observes the changes in the spectrum of light reflected from the normal untreated skin. The bands of oxyhemoglobin disappear, then reappear, only to disappear again. This may continue for some time. It is quite apparent that under these conditions readings are impossible unless one waits until a stable degree of capillary patency has been attained. Accordingly, it was found early in the development of the method that the most satisfactory readings could be made over an area of erythema. Since the first experiments were made using an arc lamp as a source of illumination, it was found that a brief exposure to concentrated light from this source produced an erythema of sufficient duration and intensity to give satisfactory results. With the development and use of an incandescent lamp as the source of light, another procedure has been adopted. After trials of several different methods, it was found that treating the skin with a one per cent histamine unction (Imadyl Uction, Hoffman-LaRoche) gave an adequate reaction which lasted throughout any of the experiments. Some few subjects are refractory and some hypersensitive, but these are rare.

The complete procedure may best be presented as a series of steps:

1. The area to be observed (usually the first interosseous space of the hand) is rubbed gently with a dry cloth and then treated with the histamine unction. The skin, of course, should be sufficiently clean to prevent artefacts from superficial substances.
2. The cuff is placed on the upper arm as in estimating blood pressure and the pressure in the tank pumped to 250 mm. Hg.
3. The excess unction is removed from the field after being allowed to remain for 2 minutes and the hand placed in position under the spectroscope. Adjustment of reflection is best made by altering the position of the hand support, i.e., half of a large cork split lengthwise. This type of support has been found the most practical of all those tried.
4. A preliminary occlusion should be made by opening the cock of the air reservoir, thus inflating the cuff. The period of occlusion should last 30 to 60 seconds.² Observation of the bands may well be made during this period to note their disappearance. Undue prolongation (60 or more sec.) should be taken as evidence of the inclusion of a vein in the region of observation and a new area should be selected.³
5. One minute after the pressure on the arm has been released the readings may be started. When possible, this one minute interval between occlusions is desirable in order

² This preliminary occlusion may be made while the ointment is on the hand, if the saving of time is a consideration.

³ A small number of individuals, constituting a very minor percentage of our observations, gave readings over one minute in length, which were confirmed by readings over other areas.

to obviate any possible effects from the preceding interruption of the blood supply. When experimental procedure demands, readings may be made more frequently than once a minute with small probability of error, but as a practice it is generally inadvisable.

6. The first reading is made as follows: simultaneously with the opening of the cock of the pressure tank, the stopwatch is started, and the time in seconds noted at the moment of disappearance of the band.

7. Check readings should be made after the usual interval. Not infrequently, in spite of the preliminary occlusion, the full development of erythema is delayed. This is manifested by a progressively increasing reduction time. Eventually it will be found that readings will stabilize. Single readings usually check within 10 per cent if proper care is exercised in manipulation and reading.

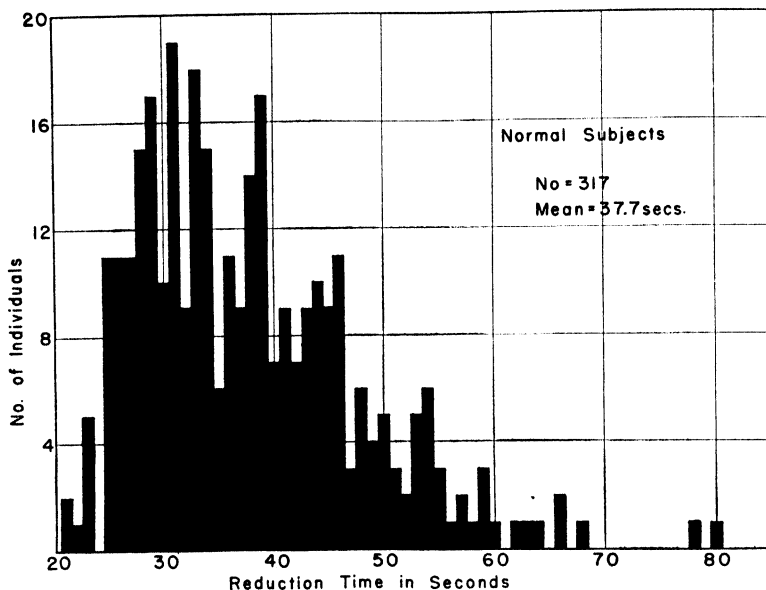


Fig. 4. Histogram showing the distribution and range of reduction time in 317 normal subjects.

Certain precautions help in obtaining stability in the readings. The subject should be in a state of rest. If he has been physically active, he should rest for 10 minutes in the position in which he is to be examined (sitting or recumbent). Furthermore, the method is sufficiently sensitive to be influenced by undue respiratory activity. For this reason, it has become routine technique to discourage conversation during the test, especially in the period just preceding or during occlusion.

Serial readings were made on a number of individuals in order to test the effect of repeated occlusions. It was found that when the subject was properly stabilized, the average of the first three readings agreed, usually within one second, with the average of ten readings.

RESULTS AND DISCUSSION. The results in a series of observations on 317 individuals are given in figure 4. Each value plotted represents an average of two or

more readings. The subjects, with few exceptions, were young men who varied considerably in habits of physical exercise. Some were medical students, others were living under conditions of strenuous physical activity. As far as this measurement is concerned, no major difference could be found between the active and the sedentary groups. It will be noted that while the range of the readings is from 21 to 80 seconds, with a mean of 38 seconds, the histogram shows certain peculiarities. The main body of readings cuts off rather sharply at 25 seconds, there being only 8 below this value. The sharpness with which the readings stop at 25 seconds suggests a well defined minimal value for the amount of oxygen available at any given moment. Had the eight subjects giving values below 25 seconds been examined carefully, some explanation might have been found. Later work on hospital cases confirms this view for, as will be reported later, short times are invariably associated with some type of dysfunction. On the other hand, times above the average are not in themselves proof of an exceptional competency of oxygen supply.

It was mentioned earlier that, theoretically, the end point of the reading occurs when the oxygen saturation of the blood reaches 20 per cent. Practically, however, the saturation is probably higher than this, owing to factors of absorption and reflection (2). A series of experiments was carried out in an attempt to estimate the saturation of the blood at the time the end point was noted. It was found, however, that at the end of the occlusion period it was practically impossible, due to circulatory stasis, to obtain enough blood for a single analysis. During the occlusion period, samples collected showed some diminution of oxygen below that of the normal sample, but this difference was far from the value calculated for the end point.

A consideration of the factors which may influence reduction time makes obvious the importance of the oxygen supply in arterial blood and the rate of utilization of oxygen. The time required for stasis to develop may also be significant. These and further factors influencing reduction time will be considered in a subsequent paper.

SUMMARY

A technique is described for the estimation of the oxygen supply to the skin. By observation of the bands of oxyhemoglobin in the light reflected from the skin of the first interosseous space of the hand, it is possible to detect a point at which a definite spectral change occurs following the sudden occlusion of the circulation. This change in the spectrum is characterized by the disappearance of the bands of oxyhemoglobin and the appearance of an apparently homogeneous illumination of the entire spectrum. The time for this change can be measured in seconds from the moment of occlusion to that of disappearance of the bands. It is spoken of as the reduction time.

This time is, in the normal subject, about 35 to 40 seconds, with a minimum of 25 seconds and a maximum of approximately 60 seconds.

The reaction appears to be related not only to the oxygen supply and its utilization, but also to the time necessary to approximate capillary stasis.

It is suggested, therefore, that the reduction time represents a complicated physiologic measurement.

Grateful acknowledgment is made to Miss Marjory Taylor for her valuable assistance in obtaining the data for this paper, and to all those who served as subjects.

REFERENCES

- (1) CANNON, W. B. The wisdom of the body. W. W. Norton & Co., New York, 1939.
- (2) EDWARDS, E. A. AND S. Q. DUNTLEY. Am. J. Anat. **65**: 1, 1939.
- (3) RAY, G. B., H. A. BLAIR AND C. I. THOMAS. J. Biol. Chem. **98**: 63, 1932.
- (4) VIERORDT, K. Ztschr. f. Biol. **14**: 422, 1878.
- (5) DENNIG, A. Ztschr. f. Biol. **19**: 483, 1883.
- (6) HÉNOQUE, A. Spectroscopie biologique. Spectroscopie du Sang. c. 1895.
- (7) MEYER, E. AND A. REINHOLD. Klin. Wehnschr. **5**: 1692, 1926.
- (8) NICOLAI, L. Pflüger's Arch. **229**: 372, 1931-32; **230**: 238, 1932.
- (9) MATTHES, K. Naun.-Schmied. Arch. **176**: 683, 1934.
- (10) MATTHES, K. AND F. GROSS. Naun.-Schmied. Arch. **191**: 39, 369, 381, 391, 523, 706, 1939.

FACTORS INFLUENCING REDUCTION TIME OF BLOOD IN THE CAPILLARIES OF THE SKIN¹

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In a preceding paper (1) an apparatus and method were described for measuring the reduction time of blood in superficial vessels of the skin. Reduction time, as the name implies, is a measure of the interval required for the reduction of hemoglobin to a constant level, after the circulation is completely occluded. Following the initial period required for stasis to develop, it is a function primarily of the rate of oxygen consumption by the tissues and the supply of oxygen available to the tissues. All physiological agencies therefore—circulatory, respiratory, nervous, metabolic, etc.—which may conceivably influence any of these variables, must be considered as possible factors affecting reduction time. A correlative study of reduction time and some of these factors was therefore made. When possible, the relationship has been expressed mathematically by means of correlation coefficients (2).

Alveolar oxygen tension. In a series of experiments on normal, healthy subjects, reduction time readings were correlated with alveolar oxygen tension values. The results are presented in figure 1, where the reduction time in seconds is plotted against oxygen tension in millimeters Hg. The points on the right, ranging from 74 to 117 millimeters oxygen tension, are those determined while the subject was breathing room air. Alveolar air was collected by means of the apparatus described by Carpenter (3), after the readings on reduction time had been made. All readings of reduction time, however, were made with the subject breathing through the apparatus since it was felt that alteration in respiratory dead space would influence the gas tensions. Those points associated with the low tensions were obtained by having the subject breath low oxygen in nitrogen mixtures, or from low pressure chamber experiments. Under these conditions the reduction time reading is the average of several taken after the body had become stabilized to the new atmosphere.

The distribution of the points shows that a definite relationship exists between alveolar oxygen tension and reduction time. Confirmation is given by the high correlation coefficient of $+0.87$.

Circulatory factors. Since upon occlusion of the arm one creates a system composed of two connected reservoirs, i.e., an arterial reservoir of high pressure and a venous reservoir of low pressure, the rate with which the blood flows from

¹A part of the work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Long Island College of Medicine.

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one reservoir to the other will depend on the difference in pressure and the patency of the connecting vessels. The excessive loss of oxygen producing the spectral change at the end point of the test can occur only when the pressure gradient between the arterial and venous sides has become so low that there is complete stasis, or at least a marked diminution in blood flow. The time taken for this equalization of pressure will be governed for the most part by the head of

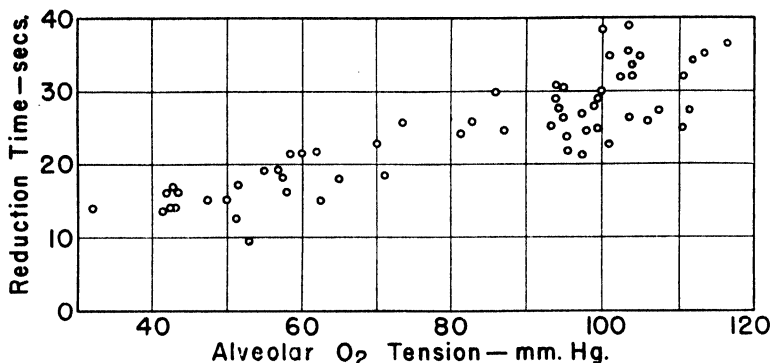


Fig. 1. Relationship between alveolar oxygen and reduction time. Linear correlation coefficient = $+0.87$.

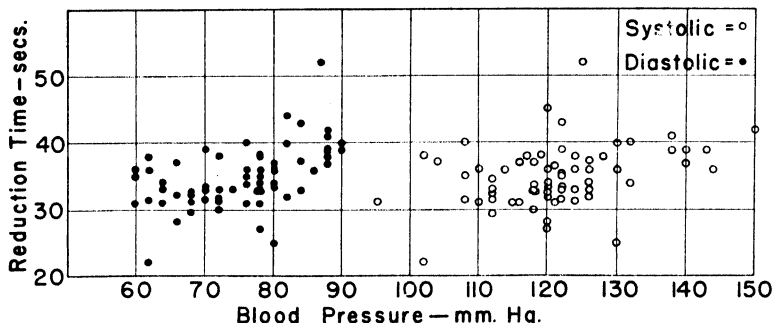


Fig. 2. Relationship between blood pressure and reduction time. The open circles represent systolic pressure; the solid circles, diastolic pressure. Linear correlation coefficients are $+0.37$ for systolic, $+0.48$ for diastolic. The subjects were taken under basal conditions.

pressure on the arterial side and by the degree of constriction of the arterioles. The best index of this head of pressure is, of course, mean arterial pressure.

Figure 2 expresses the relationship of reduction time, not with mean pressure, but with systolic and diastolic pressures. The relationship existing here is not as close as that found for oxygen tension. The correlation coefficients, however, though low, are significant in each case (2, 7), being $+0.37$ for systolic pressure and $+0.48$ for diastolic.

The importance of peripheral vascular factors on the supply of oxygen available to the tissues is evident in the following studies. In a series of eighteen experi-

ments, reduction time readings over a histamine flare produced on one forearm by an intradermal injection were compared with readings on the other arm, where an area of mild erythema was produced in the usual manner (1). Over the histamine flare, the readings averaged twice (212 per cent) as long as those over the control arm. Two exceptions were a non-reactor and another subject who overreacted with the development of a generalized flushing.

The effect of peripheral changes occurring as a result of reflex vasomotor activity was evaluated as follows: After a series of normal (or resting) readings on one hand, the subject plunged the opposite hand into hot water, or into cold water, and serial readings were continued. With vasodilatation (hot water) there was a definite increase in reduction time (16 to 25 per cent); with cold water at 10–15°C. a slight decrease (5–7 per cent) was found.

The results indicate that any undue increase in capillary dilatation will cause an increase in reduction time. Under routine conditions, where the histamine

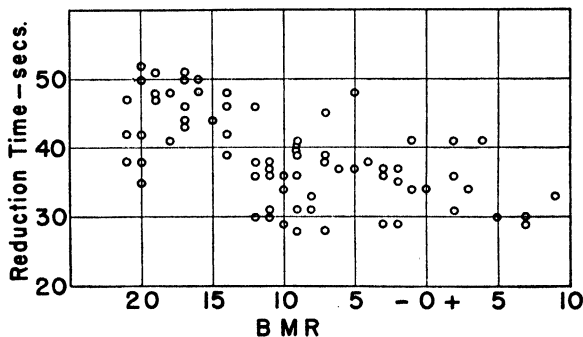


Fig. 3. Relationship between oxygen consumption (expressed as B.M.R.) and reduction time. Linear correlation coefficient = -0.60 .

unction (or any other erythema-producing agent) is used, time must be allowed for the vascular reaction to become stabilized, as indicated by consistent readings.

Circulation, therefore, particularly with respect to local vascular conditions, has a significant relation to reduction time.

Metabolic rate. The subjects used for correlating reduction time with metabolic rate came to the laboratory early in the morning and were maintained under strictly basal conditions. Duplicate determinations of oxygen consumption were made and, after an interval of about 10 minutes, the reduction time was determined. The results are given in figure 3 in which the basal metabolic rate is plotted against reduction time in seconds. The correlation coefficient of -0.60 shows a close, inverse relationship between these two variables.

Nervous factors and breath-holding. Wherever circulation and respiration are concerned, the autonomic nervous system is of possible importance. There are two ways in which this nervous influence has been studied: 1, through direct stimulation, and 2, by voluntary disturbance.

The first series of experiments involved electrical stimulation in the region of

the superior cervical ganglion. Following a series of normal readings to establish the resting reduction time, an indifferent electrode was placed on the back of the subject's neck and stimulation was applied with an alternating current of 2.9 volts, 120 cycles, by touching the skin over the ganglion with a small, stigmatic electrode. Dilation of the pupil was used as a criterion of stimulation of the ganglion. After ten seconds' stimulation, reduction time readings in the hand were made at frequent intervals. Figure 4 gives the results of the series. An initial decrease in reduction time is shown in all cases, and in nearly all there occurs a secondary decrease, before the final return to a normal, or above-normal, level.

A second group of experiments involving nervous disturbance was obtained during an extensive study of the effect of breath-holding. It was found not only that reduction time may be affected by the alteration in blood gases which occurs

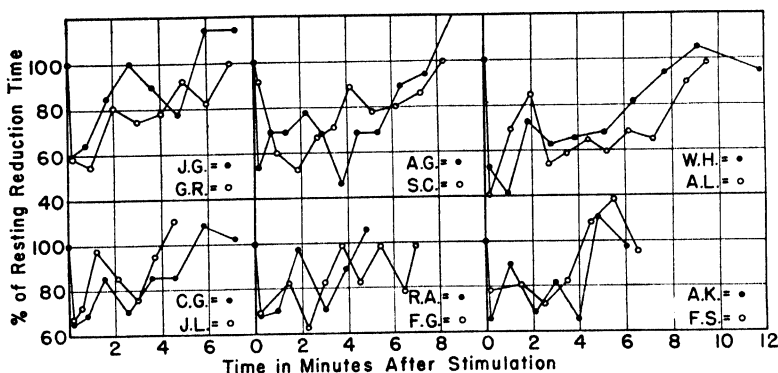


Fig. 4. Response of 12 subjects to stimulation in the region of the superior cervical ganglion. The reduction time after 10 seconds' stimulation is plotted as per cent of the average resting value.

during breath-holding, but also that there exists a nervous element in the effect produced.

The following technique was employed. After a series of resting readings, the subject was asked to hold his breath without deep inspiration, and to continue holding it until the need to breathe became imperative, but not to the point where physical effort was involved. On a signal from the subject with his free hand, his arm was occluded before a breath was taken, and the reading was made. After an interval of rest (one minute), the procedure was repeated and a check reading made.

The results in a group of 76 subjects are given in figure 5. In this series, as in most other normal subjects, the reduction time after breath-holding was definitely shorter than that found for the resting state. The average decrease, expressed as percentage of the normal resting value, is 20.4 per cent, with extremes of zero and 40 per cent.

The presence of a nervous element affecting the terminal limit of breath-holding is well recognized (4, 5, 6). If this nervous element is a manifestation of the

stress of holding the breath, its effect on reduction time might be demonstrated early in the breath-holding period, before there could be an appreciable change in blood gases. Accordingly, in a series of 71 experiments, after the effect of breath-holding on reduction time was determined, the subject was asked to hold his breath and occlusion was made at only 10 per cent of his previous breath-holding time, that is, after only 2 to 6 (in one case 7) seconds. After occlusion, normal respiration was resumed. The readings made in this manner were found to be as short as, or shorter than, those made at the limit of the breath-holding period. When occlusion was made at the terminal limit of breath-holding, the average decrease in reduction time was 17.3 per cent; for the ten per cent period, the average decrease was 20.2 per cent.

Conscious inhibition of breathing thus produces bodily effects which extend far beyond the respiratory system. The prompt change in reduction time on the initiation of breath-holding can be explained only on the basis of a generalized

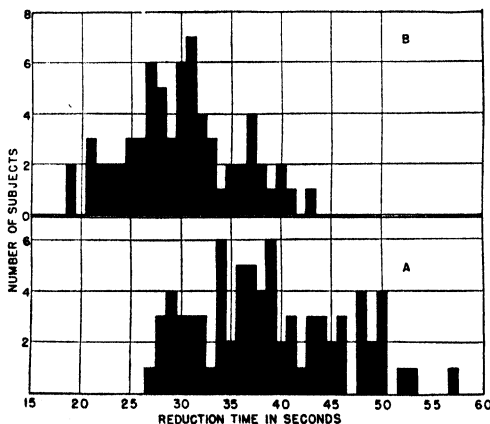


Fig. 5. Histograms showing the effect of breath-holding on reduction time. A, normal distribution under basal conditions, (average time 39 sec.); B, distribution after breath-holding (average time 29 sec.).

neurogenic reaction manifested primarily by peripheral vascular changes. Changes later in the breath-holding period are believed to be influenced by the lowering of oxygen tension and the accumulation of carbon dioxide. All of these factors may be in action when occlusion is made during the uncomfortable period near the terminal limit of breath-holding, and must be taken into consideration in studying the effect of breath-holding on reduction time.

SUMMARY

Reduction time, i.e., the interval required, after occlusion of the circulation, for the reduction of hemoglobin to a constant level, is a function of many physiologic variables, particularly alveolar oxygen tension, systolic and diastolic blood pressures, peripheral vascular conditions and metabolic rate. The linear correlation coefficient (r) between reduction time and some of the variables studied is as follows:

Alveolar oxygen tension.....	$r = +0.87$
Systolic pressure.....	$r = +0.37$
Diastolic pressure.....	$r = +0.48$
Basal metabolic rate.....	$r = -0.60$

The value of r for alveolar oxygen and reduction time is of sufficient magnitude to indicate almost complete correlation. Metabolic rate shows a marked inverse relationship.

Systolic pressure has the lowest value of all those computed, and the value of diastolic pressure is not much greater, but they both actually fall into a category recognized as being definitely significant (2, 7). Evidence of the rôle played by the smaller vessels in the supply of oxygen to the tissues under observation is given by studies of histamine flares, and the reflex effect of heat and cold.

As suggested by the importance of circulation and respiration as determining factors of reduction time, nervous disturbances are found to have a profound effect. It has been shown that stimulation in the region of the cervical sympathetic ganglion produces a marked decrease in reduction time, both immediate and prolonged. A similar immediate decrease is also observed when the breath is held.

A period of breath-holding maintained to a point of discomfort produces in normal subjects a decrease in reduction time which is believed to be a result of possible alterations in all of the factors reported in this paper.

Grateful acknowledgment is made to Miss Catherine Elias, Mr James H. Ray and Miss Marjory Taylor for their valuable assistance in obtaining the data for this paper and to the many students and staff members who served as subjects.

REFERENCES

- (1) RAY, G. B. This Journal **147**: 622, 1946.
- (2) DAVENPORT, C. B. AND M. P. EKAS. Statistical methods in biology, medicine and psychology. John Wiley & Sons, New York, 1936.
- (3) CARPENTER, T. M. J. Nutrition **4**: 281, 1931.
- (4) DRAPER, G., C. W. DUPERTUIS AND J. L. CAUGHEY, JR. Human constitution in clinical medicine. Paul B. Hoeber, Inc., New York and London, 1944.
- (5) SCHNEIDER, E. C. This Journal **94**: 464, 1930.
- (6) WHITE, P. D. Am. J. Med. Sci. **159**: 866, 1920.
- (7) GUILFORD, J. P. Fundamental statistics in psychology and education. McGraw-Hill Book Co., New York and London, 1942.

THE CHANGE IN REDUCTION TIME OF BLOOD AFTER BREATH-HOLDING AS A CRITERION OF PHYSIOLOGICAL FITNESS¹

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Experiments cited in a foregoing paper (1) indicate that reduction time, i.e., the time required after occlusion of the circulation for the reduction of hemoglobin to a constant level, depends upon the associated reactions basic to the supply of oxygen to the tissues. Since constitutional adequacy is commensurate to some extent with the maintenance of an adequate supply of oxygen to the regions of essential activity, reduction time measurements may conceivably have some relation to the efficiency of the body in this respect, especially if they include measurements of changes observed in response to a stress. Such a stress may occur during voluntary breath-holding, particularly if carried to a point of discomfort. Breath-holding has a possible effect on peripheral circulation through changes in oxygen and carbon dioxide tensions (2), and it is known to produce a change in reduction time (1). If this influence is manifested as a general cutaneous constriction or dilatation, it represents a change in blood supply to the surface and is indicative of a reciprocal change in supply elsewhere at more vital centers. We have, therefore, in the response to breath-holding, a possible measure of the degree of homeostatic adjustment to a condition of stress.

If the above reasoning is true, one would expect to find a relation between the change in reduction time produced by breath-holding and fitness, considered from a physiological standpoint. In order to establish the validity of such a relation, several groups of individuals have been studied, comprising men of various recognized degrees of fitness. For comparing individuals and groups, a system of scoring has been established, based upon the change in reduction time on breath-holding. The score may be defined as the decrease in reduction time expressed as a per cent of the resting value, and computed mathematically by the formula:

$$\frac{\text{Resting Reduction Time minus Reduction Time After Breath-holding}}{\text{Resting Reduction Time}} \times 100$$

Thus, the greater the percentile decrease in reduction time, the higher the score, and if the reduction time shows an increase after breath-holding, the score becomes a negative value.

The first group of subjects to be studied was made up of first year medical

¹ The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Long Island College of Medicine.

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students on a normal, peacetime schedule before the accelerated program was put into effect. The results, expressed in the form of a histogram (fig. 1 A), show a mean value of 20.4 for the reduction time score. Compared with this is the score of 14.7 (fig. 1 B) found in a second group of medical students two years later, who had been working for two years under the strain of an accelerated wartime schedule. Nearly all of these students had been accepted for military or naval service, and were therefore considered to be physically adequate. A few civilians were used when the reason for rejection from service did not involve a major physiological discrepancy.

During this work, and in later studies on men in training, it was repeatedly observed that subjects who had a cold gave a small decrease on breath-holding (less than 10 per cent) or no change whatever. After recovery from the cold, the scores would fall somewhere in the zone between 10 and 35. Such observa-

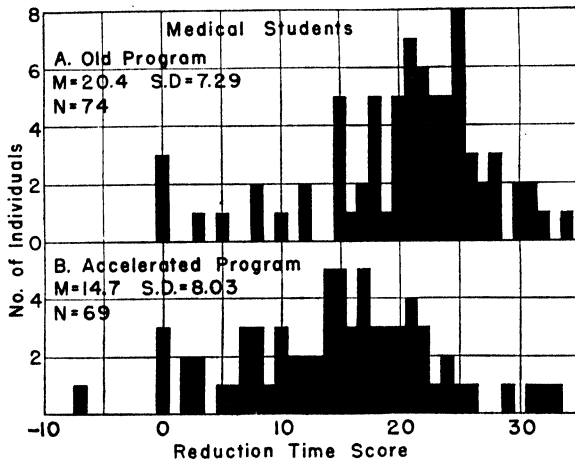


Fig. 1. Distribution of reduction time scores for two groups of medical students. $M.$ = mean score, $S.D.$ = standard deviation, $N.$ = number of students in the group.

tions were so frequent, not only for colds but for other conditions which would be considered physiologically abnormal, that a score of 10 came to be considered as a borderline between fitness and lack of fitness of the subject. Thus a higher score is associated with a greater degree of fitness.

When readings were undertaken on hospital patients, this view was substantiated. It was found that individuals who were bedridden and clinically ill gave scores not far from zero, or often on the minus side. The aggregate results of 226 observations on patients where some abnormal condition was clinically active are presented in figure 2. In some cases, particularly in trauma, it is understandable that a patient might be physiologically fit and at the same time mechanically unfit. The patients included in this chart were all ill enough to be confined to bed, or, if ambulatory, were included only when the clinician in charge indicated that the patient could not be classified as truly convalescent. They include traumatic, respiratory, cardiac, nervous and infectious cases. The mean

of 226 observations is a score of -13.5 . The standard deviation is high as compared with the normal groups, but this is to be expected in a series where the subjects are by no means uniform in condition. Some were severely ill, while others were at the borderline between illness and convalescence.

In figure 3 a histogram of wide distribution characterizes a mass series of observations on persons who could be classed as convalescents. These subjects came from a variety of sources, and ranged from men who were considered employable, but still under medical supervision, to patients just out of bed in the hospital. The employed subjects, 22 in number, were in the training school of a large war plant, having been discharged from the armed services for various medical reasons. Another portion of the group, comprising 46 subjects, were convalescent seamen at a Merchant Marine Rest Center. Many of these two

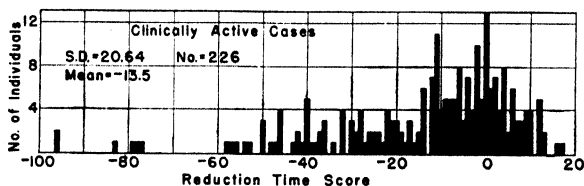


Fig. 2. Distribution of reduction time scores in 226 observations on hospital patients who have an active clinical condition.

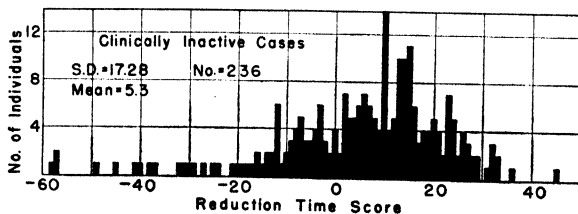


Fig. 3. Distribution of reduction time scores in 236 observations on convalescents and men discharged from service for physical or nervous disability.

groups gave readings falling within the area we have designated as normal. Among the hospital patients, a study of convalescence is difficult. In many cases, patients were allowed to leave the hospital before true recovery because home conditions were such that convalescence could be entrusted to them. On the other hand, some subjects remained in the hospital for periods beyond the return to normal health, to a point where a softening-up process occurred. The average score of 5.3 for the entire group has little meaning, because of the wide distribution, covering the fields both of normal and clinical subjects, and because of the wide range of conditions which characterize the components of the group.

The results given so far show that there is a marked difference in the general picture presented by the clinically ill as compared with medical students in normal health. These students, particularly those who are carrying an accelerated program where there is little time for physical exercise, represent a seden-

tary type of person. In order to correlate reduction time with fitness as demonstrated by performance, a group of Marines was studied. These men were chiefly non-commissioned officers detailed to special classes for advanced training. A few commissioned officers and enlisted men were included, but the group as a whole was composed of men who had shown superior physical qualities and were in all ways fit for the vigorous training they had undertaken.

The aggregate results of the observations are presented in figure 4. The mean score of this group is 29.9, as compared with 20.4 and 14.7 for the student groups, and -13.5 for the clinically active group. Attention should be directed to similar distributions of the three sets of data on normal persons, as shown by the similarity of the standard deviations (7.29, 8.03 and 8.35).

There can be no question as to the effect that physical training has on the reduction time score. When the total group is broken down into its component groups, the effect of the rigorous field training becomes even more apparent. The total was composed essentially of four groups, representing three stages of training. It should be understood that training, in the sense used here, does not mean elementary or basic training, but strenuous advanced work. The four groups had the following histories:

Members of the Machine Gun School had been drawn from many replacement areas, and as a result, their backgrounds varied. They had just started training, i.e. were in their first week.

Members of the First Sergeant's School had all been in classes for the preceding three months where little activity was possible. They, too, were in the first week of training.

Men in the Rifle School were again from various sources. They had had two weeks of advanced training.

The final group, men in the Weapons School, included not only the non-commissioned officers, but a number of commissioned instructors as well. Those receiving training had had nine weeks of rigorous work. On the basis of our readings it was apparent that the instructors were in the same stage of fitness as the men under them. They have, therefore, been considered together.

The result of breaking down the data of figure 4 into its component groups is given in figure 5. It will be seen that the distributions fall into three categories. The mean scores found for the first two groups, the Machine Gun School and the First Sergeant's School, are very close, 26.8 and 27.2 respectively. These two groups, it will be recalled, were in the first week of training. Although the past histories varied, the degree of fitness of each appears to be about the same.

Comparison of these men with those in the Rifle School shows a somewhat greater difference in mean scores. The difference between 27.2 and 30.4 is not great, but statistically it is significant (3). At the time of the test, these groups were living under identical conditions and were of more or less similar backgrounds and original training. It is judged, therefore, that the one week of additional advanced training was probably the major factor in the difference of results.

The group from the Weapons School definitely represents a different category from the other three groups. The mean score for this group is 36.0, as compared

with a mean of 28.3 for the entire group of 140 men in their first two weeks of training, or a mean of 30.4 for the nearest group (Rifle School).

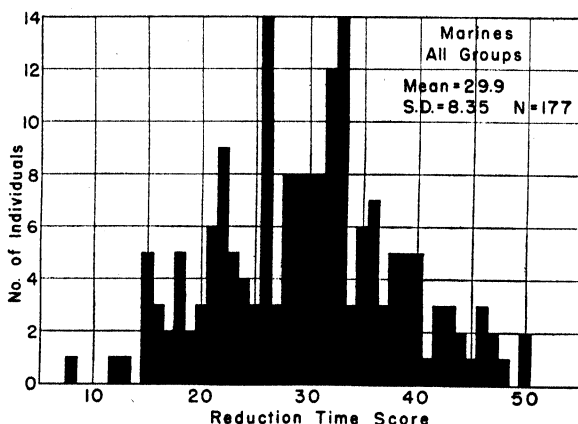


Fig. 4. Distribution of reduction time scores of 177 Marines on active duty

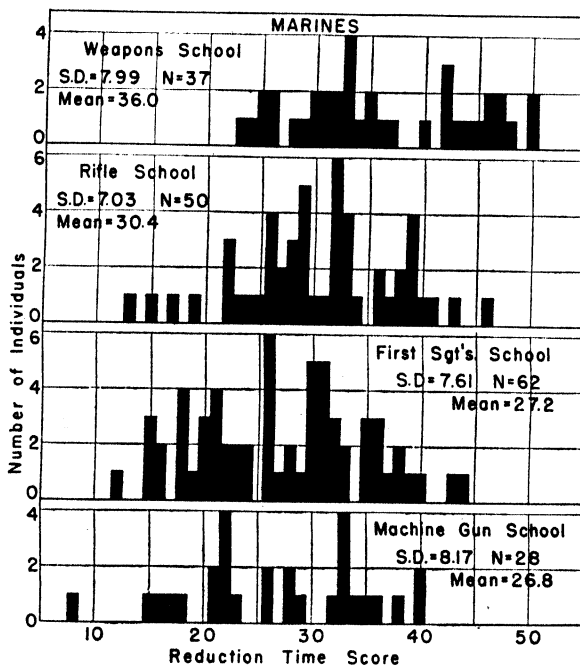


Fig. 5. Histograms showing the influence of training on reduction time score. Explanation in text.

If we add the results in figure 5 to those in figure 1, obtained on medical students, we have a range extending from a group on a restricted physical schedule to a group where physical activity is very strenuous and has been of sufficiently

long duration for all members to be in top condition. It will be seen in this range that the average reduction time score increases progressively with increasing fitness, from a minimum of 14.7 to a maximum of 36.

A further series of readings was made on 92 officers and men at a Naval Air Station. These men were engaged in flying or in otherwise maintaining a heavy schedule which precluded a degree of organized activity comparable with the Marines in training. About two-thirds of these subjects were pilots or rear gunners and the remainder were concerned with the operations. The readings on flight personnel included here were taken in the morning, before the day's

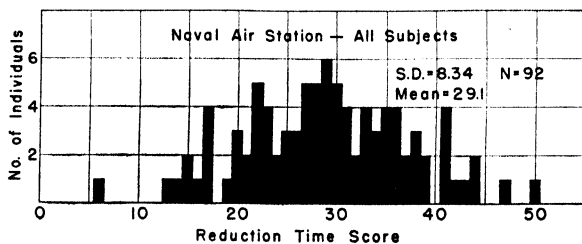


Fig. 6. Distribution of reduction time scores of 92 subjects at a United States Naval Air Station.

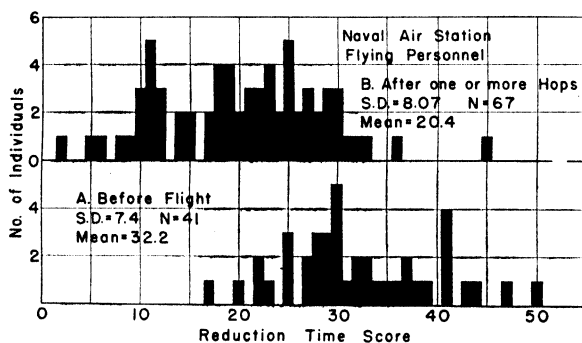


Fig. 7. Histograms showing the effect of one or more dive-bombing flights on reduction time score.

flying activity started. The mean score for the group (fig. 6) is 29.1, as compared with 29.9 for the Marines. It would seem as though these two groups might well be considered as our standard for young men. We have a total of 269 observations which show that for healthy young men living under adequately active conditions the average reduction time score is 29 to 30.

The Naval Air Station personnel can be broken down into flying and non-flying groups. As might be imagined, the scores of the 41 aviators, at rest, were distinctly above those of the group as a whole. The data for this group are presented in figure 7 A. The mean score is 32.2, which is well above that for the average Naval or Marine subject. These men were dive-bombers in the final stage of training, where it is to be expected that only the fittest had survived the

long arduous training. They maintained a routine of one hour a day at some form of organized physical activity and were without question a superior physical type. Yet this group of men showed changes after flight which, in our system of scoring, were definitely indicative of a loss in fitness. These changes are represented in figure 7 B. The graph gives the results of 67 observations, made chiefly on pilots, but including a few on rear gunners, after one or more dive-bombing flights. The distribution has shifted toward the less fit region, the mean score being 20.4 as opposed to the pre-flight score of 32.2. Furthermore, many of the readings approximate, or fall in, the unfit zone. An occasional pilot would show little change from his pre-flight reading, but these men were the exception rather than the rule.

That this falling off of the score after flight was not due to failure of the physiological compensatory or adaptive reactions, but rather to the fact that these

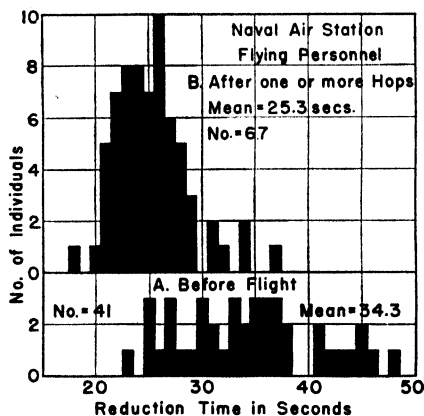


Fig. 8. Histograms showing the change in resting reduction time as a result of one or more dive-bombing flights.

reactions were already working before the breath was held, is shown in figure 8, where the decreased score after flight is seen to be due to a shorter resting reduction time rather than to a longer reduction time after breath-holding. In fact, the readings after breath-holding also tended to drop, but not to the same extent as the resting readings. The alteration was noticeably progressive as the day went on, with the result that, after the final flight of the day, many men were in the zone where fitness was definitely questionable. The reaction was, beyond a doubt, the result of the nervous strain of flying the heavy planes under the necessary rigorous conditions of this stage of training, rather than the result of the muscular effort of flying, since the rear gunners showed the same, or an even greater degree of effect. Equally significant was the prompt return to normal readings after a night's rest; nearly every man who had had a proper sleep returned to his normal reduction time score when he reported to the Ready Room at 0730 the next morning.

In comparing the results given in figure 8, showing the change in resting re-

duction time after flight, with those showing the effect of stimulation in the region of the cervical sympathetic ganglion (1), it is suggested that the fatigue shown by pilots and gunners after flight may be a nervous reaction with a definite autonomic representation. The importance of the nervous element in any evaluation of physiological fitness should be recognized and considered as one of the factors qualifying the estimation.

Although exercise and training have an obvious influence on the fitness of an individual as scored by the reduction time test, it is by no means a test of performance. We have found individuals of excellent performance in specific tests who scored poorly in reduction time. Not always has a cause been found for this lack of correlation. Occasionally, however, an individual of this type is known to be one who performs well when he is physically and mentally master of the situation, but who is liable to break down under adverse circumstances. Through practice, a potential anxiety neurotic may acquire a trick of performance, but when such an individual is confronted with a situation involving tension, a distressing state of physiological disability may develop because of an abnormal pattern of adjustment.

As an illustration, it may be mentioned that prior to the readings made on the Marines, a request had been made of the collaborating medical officer that some individual of known unfitness be included with the normals to ascertain whether it was possible for the method to pick him out of the whole group. This man, found later to be a manic depressive, was detected at once. His resting reduction time was 26 seconds, which is definitely low compared with the others of this group, and there was no change on breath-holding. In fact, of the total 190 observations made in the series, his was the only reading which showed no decrease on breath-holding.

Because of the sensitivity of the readings to the passing condition of the individual, it was decided to test the reliability of the method in a manner suggested by Guilford (4). Such a study involves making readings on the same individuals on two separate occasions, and finding the correlation coefficient of the two series of readings.

In this correlation study a group of volunteer medical students was used. The conditions under which the test was made were controlled to a reasonable extent, in an effort to make them as uniform as possible. The student was tested in the morning before his first class, on two separate days not more than a week apart. He was asked to come in a rested, normal condition, and not to report for the second test if he had a cold, or felt that his condition, physically or nervously, was not comparable with that of the first morning.

The result of this experiment gave a correlation coefficient of $+0.81$. In the course of our studies, we have come to a belief that with our method of scoring, a value may have a limit of accuracy of approximately five points, that is, about 2.5 in either direction, since in calculating the per cent change a variation in reading of one second may result in a variation of from two to five points in the resulting score. With this in mind, the test-retest correlation coefficient of $+0.81$, where 78 per cent of the values obtained in the second test were

within five points of the corresponding values in the first test, would seem to show a good degree of reliability, in spite of the great variation in a few instances.

DISCUSSION. A tendency has always existed to regard "physical fitness" and "fitness" as synonymous terms and to use them interchangeably. Physical fitness, however, should have a much more limited meaning and should be used only when describing or defining the physical adequacy of a system. In the human body physical fitness should be measured by such factors as body build, muscle mass, skeletal structure, tissue strength, lung capacity, etc. These physical or structural qualities form the basis of true physical fitness. They are, of course, significant factors in the determination of fitness considered from any angle but, as it is ordinarily interpreted, and certainly as it is evaluated in the human subject, fitness has essentially a functional or physiological basis and should therefore not be referred to as "physical." We suggest that the term "physiological fitness" be considered as a much more meaningful expression for use in this sense.

A number of different tests have been designed for measuring fitness, some based upon performance, some upon reactions and adjustments under autonomic control, and some upon a combination of these two. Performance tests are usually regarded as unreliable because of their dependence upon such factors as knack, practice, incentive, co-operation and willingness on the part of the subject, etc. There is, however, no adequate basis for comparison of different fitness tests for, as Schneider (5) points out, fitness has not regularly been applied to any particular state or condition. Many people regard fitness as synonymous with health. It is frequently associated with ability to do work, or to endure exercise without fatigue, and it is generally agreed that fitness increases with training. But to find a simple test for evaluating every phase of fitness in its broadest aspect is probably impractical, if not impossible. Some of the recent work at the Harvard Fatigue Laboratory has been directed toward developing tests suitable for measuring a specific phase of fitness, such as fitness for hard muscular exercise. Another test might be specific for measuring one's fitness for high altitude flying. Still another might determine fitness for working under conditions of great nervous tension such as would be encountered during wartime. In all of these tests, however, a high score depends not only upon training or development along a certain line, but also upon the integrity and adaptability of certain basic physiological reactions.

Our adoption of the reduction time score as a yardstick for measuring fitness is based not only upon the high degree of correlation which we have found between the score and recognized fitness in various groups, but also upon our own conception of the physiological mechanisms involved in the test. The skin, as well as certain other parts of the body, normally acts as a storehouse for blood. The volume circulation through cutaneous areas and the actual capacity of the small vessels of the skin may vary tremendously under changing conditions of the internal and external environments without, apparently, interfering with the normal metabolic processes or other functions of the skin. Normally, at rest, a portion of the blood present in these small vessels, including the capillaries, is not necessary from the standpoint of the integrity of the skin itself, but repre-

constriction after holding his breath to the point of discomfort. By the same line of reasoning, anyone with a reduction time score appreciably less than 10 to 12 probably has some degree of dilatation of the skin vessels. Regardless of how much or how little effect the decreased oxygen content of the blood may have on the score, its relative value as a criterion of physiological fitness, on the basis of cutaneous capillary changes and redistribution of blood under conditions of stress, remains the same.

SUMMARY

The change in reduction time after breath-holding is described in its relation to physiological fitness. In general, after a period of breath-holding, reduction time shows a decrease in normal persons, and either no change or an increase in persons who are ill. For comparative purposes, the per cent decrease on breath-holding is used as a score, a greater score signifying a greater degree of fitness.

Readings made on groups of various recognized degrees of fitness gave scores that ranged from 36.0 (mean score for a group of Marines) to -13.5 (mean score for hospital patients).

In a study of flying personnel at an air station, a change in score indicating a decrease in fitness after one or more flights is interpreted to mean that physiological compensatory reactions are at work, as a result of the nervous strain of flying.

A test-retest study gave a correlation coefficient of +0.81.

The decrease in reduction time after breath-holding is believed to be an indication of the adjustment of the body to a condition of stress. The primary factor involved in this change is the shifting of blood from the skin region to other parts of the body.

Grateful acknowledgment is made to Dr. S. P. Bartley for his co-operation in obtaining the data on hospital patients; to Miss Catherine Elias, Dr. Frances Greenwood, Miss Susanne Latson, Mr. James H. Ray, Dr. Daniel J. Sullivan and Dr. Clarence Wasmund for their valuable assistance in obtaining experimental data; to those officers in the Medical Field Research Laboratory of Camp Lejeune, North Carolina, in the Naval Air Station at Wildwood, New Jersey, in the Merchant Marine Rest Center at Oyster Bay, New York, and in the Brooklyn Naval Hospital, Brooklyn, New York, through whose co-operation many valuable data were obtained; and to the many students, patients, service personnel and others who served as subjects.

REFERENCES

- (1) RAY, G. B., L. H. RAY AND J. R. JOHNSON. *This Journal* **147**: 630, 1946.
- (2) GELLHORN, E. *Autonomic regulations*. Interscience Publishers, New York, 1943.
- (3) DAVENPORT, C. B. AND M. P. EKAS. *Statistical methods in biology, medicine and psychology*. John Wiley and Sons, New York, 1936.
- (4) GUILFORD, J. P. *Fundamental statistics in psychology and education*. McGraw-Hill Book Company, New York and London, 1942.
- (5) SCHNEIDER, E. C. *Physiology of muscular activity*. W. B. Saunders Company, Philadelphia and London, 1939.
- (6) MEAKINS, J. AND H. W. DAVIES. *J. Path. and Bact.* **23**: 451, 1920.

sents a reserve supply for use in other parts of the body as the need may arise. This might well be called the "peripheral circulatory reserve." During breath-holding, as well as during other forms of body stress, this peripheral reserve is called upon to increase the blood supply to other areas, such as the brain and heart, which are more sensitive to a decrease in oxygen supply. Gellhorn (2) has described this sort of autonomic adjustment in the body as evidenced by plethysmographic records of decreased arm volume during breath-holding. It constitutes an important phase of Cannon's emergency reaction and is one example of how the body adapts itself physiologically to stress by means of a sudden redistribution of its supply of oxygen.

It is this redistribution of oxygen—this shifting of blood from the skin areas to other regions—which we believe to be the primary factor responsible for the decrease in reduction time normally seen after breath-holding. Since reduction time is a measure of the interval required for the hemoglobin in the blood to become reduced to a constant level during complete occlusion of the circulation, the only other factors which might possibly be concerned are: 1, the time for stasis to occur in the capillary bed under observation, 2, the rate at which oxygen is utilized from the blood, and 3, the oxygen content of the blood as it reaches the capillaries.

The interval of time between occlusion of the circulation and complete stasis is a function of the arterial and venous pressures and the resistance offered by the small intervening vessels. Pressure changes during breath-holding as carried out in these experiments were negligible, and the constriction of small vessels which undoubtedly occurs would tend to prolong stasis time rather than shorten it. The effect that any alteration in this factor might have on total change in reduction time would at most be only slight, and for all practical purposes it may be disregarded.

The rate of oxygen utilization from the blood is a direct function of the metabolism of the surrounding tissues, in this case skin. This again is an unknown quantity in determining reduction time, but a priori there seems little chance of its being appreciably altered during breath-holding. According to Gellhorn (2) the metabolism of tissues decreases under the influence of an increased CO_2 tension in the blood, thus tending to increase the reduction time. Since reduction time normally decreases as the breath is held, we may conclude that it is not due to a change in the rate of oxygen utilization.

The importance of blood oxygen content as a factor in determining reduction time is indicated by the high correlation coefficient found (1) between alveolar oxygen tension and reduction time. The extent to which the oxygen saturation of the blood decreases during a period of 25 to 45 seconds' breath-holding³ is probably in the neighborhood of 10 per cent and not exceeding 15 per cent. Meakins and Davies (6) reported an oxygen saturation of 83.8 per cent at the end of 40 seconds' breath-holding, which is a decrease of approximately 12 per cent below normal resting values. On this basis we might suggest that anyone with a reduction time score exceeding 10 to 12 shows evidence of a cutaneous capillary

³ The range within which most of our values for normal subjects fell.

EXPERIMENTAL HYPERTENSION IN THE DOG¹

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Earlier studies on experimental hypertension led to the almost universal acceptance of the idea that chronic hypertension as observed in the experimental animal and presumably also in the human was a result of the liberation of a pressor substance by the ischemic or otherwise injured kidney. The inadequacy of this view has, however, been amply demonstrated by more recent work (9, 10). Most of the recent work has been performed on the rat and rabbit instead of the dog, which was the animal used for the experiments on which the earlier conclusions were based. It was deemed desirable, therefore, to reinvestigate the problem on the latter species, for, if we assume that experimental chronic hypertension is analogous to that seen in the human patient, any theory advanced to explain its pathogenesis must be applicable to all laboratory animals.

The present paper concerns itself with the study of the effects of various manipulations on the kidney of the dog similar to those previously recorded on the rat and rabbit. It is based on the study over a period of five years of over 50 dogs. The data presented aid to elucidate the pathogenesis of hypertension and correlate the findings with those previously noted in other species. They indicate that there is no essential difference in the reaction of the rat, rabbit or dog and that many conclusions based on earlier work on the dog require modification.

METHODS. Hypertension was induced by applying a figure-of-eight ligature to the kidney by the method described previously (6) instead of by the use of the Goldblatt clamp applied to the renal artery as used by earlier investigators. This procedure has been found to have certain advantages over the use of the clamp, for it does not induce necrosis of the kidney (such as occurs when the clamp is too tightly applied) and results in a more slowly developing chronic type of hypertension. The immediate rise in blood pressure which occurs following the application of a clamp is not noted usually following the application of a figure-of-eight ligature and, as shall be shown later, it is this early rise in pressure which has led to erroneous conclusions regarding the nature of chronic hypertension.

The blood pressure was determined directly by puncture of the femoral artery with a needle attached to a mercury manometer. After a short period of training, this can be done without exciting the animal and without the use of an anesthetic. Readings, except where otherwise indicated, were made two to three times weekly.

The animals were selected for their docility from ordinary mongrels supplied

¹ Aided by a grant from the John and Mary Markle Foundation.

to the laboratory and were of medium size (6 to 12 kgm.). They were fed with a commercial dog food except during periods when subjected to dietary experiments.

All operations on the kidney were performed under ether anesthesia through a lumbar approach. The various types of operation performed are described below.

Analyses of oxalated venous blood were carried out by the following procedures: carbon dioxide combining power, method of Van Slyke and Stadie (22); sodium, method of Butler and Tuthill (3); chloride, method of Van Slyke (21).

RESULTS. To induce chronic hypertension of a reasonable degree in the dog it is necessary to apply the figure-of-eight ligature to one kidney and remove the other. Unlike the rat and the rabbit, where ligature of one kidney without

TABLE 1

The effect of various experimental procedures on the mean blood pressure of the dog

All the results are expressed in terms of millimeters of mercury. The post-operative values are averages obtained during the period of three to six months following the experimental manipulation indicated in the third column.

EXPT. NO.	NUMBER OF ANIMALS	OPERATIVE PROCEDURE	AVERAGE MEAN BLOOD PRESSURE	
			Before operation	After operation
1	15	Constriction of one kidney; other remaining intact	127	145
2	12	Removal of one kidney; other remaining intact	125	140
3	10	Removal of normal kidney after previous constriction of other kidney	140	170
4	10	Constriction of remaining kidney after previous unilateral nephrectomy	138	169
5	6	Removal of previously constricted kidney; other kidney intact	142	145

contralateral nephrectomy results in hypertension in an appreciable percentage of animals (12), the effect in the dog is less striking as shown below. Following application of the figure-of-eight ligature there is usually no rise in blood pressure immediately nor on the days following the operation. It is only after some weeks that any effect on the blood pressure becomes evident. This is unlike the result seen after constricting the renal artery in which case there is an immediate rise in blood pressure which gradually disappears during the subsequent weeks (1, 5, 19, 23). The chronic stage of hypertension develops without this transient preliminary rise when the figure-of-eight technique is used and differs in its pathogenesis from the elevation seen soon after the application of the clamp.

Effect of unilateral constriction or nephrectomy. As shown in table 1, the constriction of one kidney (expt. 1) or its removal (expt. 2), the other kidney remaining intact, results in a slight but definite rise in blood pressure after three to six months have elapsed. The effect in different animals varied from 0 to 50 mm.

with an average rise of 18 mm. in the case of constriction, and 15 mm. in the case of nephrectomy. The difference between these two is not significant. However, the fact that a slight rise occurs following either operation appears to be definite. The failure of previous workers to detect the rise in pressure following unilateral nephrectomy must be attributed to their not having followed the blood pressure long enough following operation and the fact that the rise is so slight as to be overlooked unless frequent readings are taken over many weeks.

It is significant that nephrectomy exerts the same effect as does constriction of the kidney. Either operation apparently interferes with some normal function with the same consequence insofar as the blood pressure is concerned (8).

Effect of a subsequent nephrectomy or constriction. As seen in table 1 (expts. 3 and 4), nephrectomy or constriction of the remaining kidney after either of these manipulations has been performed on the contralateral kidney, results in a further increase in blood pressure which is approximately twice as great as that which followed the first operation. Apparently the remaining normal kidney compensates to some extent for the damage inflicted on the other organ by the first operation. The second operation therefore induces a more pronounced effect because it can no longer be counteracted by normal renal tissue.

The blood pressure three to six months following the constriction of one kidney and removal of the other is usually between 160 and 180. In some instances it may be as high as 190 to 200 and in about 5 per cent of the animals the blood pressure remains within relatively normal limits despite the operations.

Effect of removal of a constricted kidney. As seen in experiment 5 of table 1, removal of a constricted kidney does not abolish the pre-existent hypertension induced by a unilateral operation. Where only one constricted kidney remains, its removal also fails to cause a drop in the blood pressure which remains at its preoperative level for several days and falls only as uremia and death approach. This is illustrated in table 2 where the results on 3 dogs in which hypertension had been present for a year were subjected to a removal of a remaining constricted kidney, the other kidney having been removed over a year previously. In order to avoid the shock and trauma of the operative procedure, the adhesions surrounding the constricted kidney were freed in a preliminary operation and the kidney transplanted to the flank. Several days later it was removed.

Effects of therapeutic measures in reducing the blood pressure. Among the therapeutic measures effective in lowering the blood pressure in experimental chronic hypertension in dogs, the use of renal extracts administered orally has been reported previously (20). Of other measures used experimentally and in the human, the most effective are sodium deprivation (11, 13) and the oral administration of certain oxidized oils (7). These have also been applied to the dog. The effectiveness of oil extract in reducing the blood pressure in the dog has been reported previously (7). The time required for the oil to be effective is intermediate between that observed in the rat (30 to 50 hrs.) and the human (several weeks). As in both the rat and the human the pressure is not reduced to normal but to a level which depends upon the height and duration of the initial pressure.

Drastic reduction of the sodium intake of the hypertensive dog failed in nine

animals to reduce the blood pressure. The animals were maintained for a month on a diet composed of a mixture of dialyzed milk, rice and salt-free butter to which supplements of crystalline thiamine, riboflavin, and nicotinic acid were added. Despite the fact that this diet is exceedingly low in sodium content, no perceptible drop in blood pressure occurred. In three animals, ammonium chloride in doses of 3 grams daily was added after they had been maintained on the low sodium diet for a month. This resulted in a drop in blood pressure and death in acidosis in two of the three animals after therapy for one week. The sodium-depleted dog is apparently highly susceptible to the acidotic action of ammonium chloride.

TABLE 2

The effect of removing the remaining constricted kidney on the blood pressure of previously unilaterally nephrectomized dogs

	EXPERIMENT 1	EXPERIMENT 2	EXPERIMENT 3
	Mean blood pressures in millimeters of mercury		
Prior to final nephrectomy.....	200	190	185
One hour post-operatively.....	180	180	190
Three hours post-operatively.....	200	190	190
Eight hours post-operatively.....	190	180	180
24 hours post-operatively.....	180	185	185
32 hours post-operatively.....	160	170	180
48 hours post-operatively.....	Dead	150	170
56 hours post-operatively.....		Dead	160
72 hours post-operatively.....			Dead

TABLE 3

*The sodium, chloride and carbon dioxide combining power of the blood in normal and hypertensive dogs**

	SODIUM	CHLORIDE	CO ₂ COMBINING POWER
	milliequiv. per liter	milliequiv. per liter	mm. of mercury
Normals.....	153 \pm 3	98 \pm 5	56 \pm 6
Hypertensives.....	155 \pm 5	95 \pm 7	59 \pm 4

* Analyses by Miss Earline Milstead.

The failure of the hypertensive dog to react to sodium restriction was unexpected since the same procedure has a remarkable reaction both in the rat (11) and in an appreciable percentage of human hypertensives (13). The fact that the procedure is not effective in the dog nor always effective in man (13) indicates that hypertension is not always simply a result of sodium retention. The failure of the hypertensive dog to respond in any case might be attributed to the fact that in this species there is no retention of an excessive amount of sodium in hypertension. To test this hypothesis, the sodium and chloride contents and the carbon dioxide combining power of the blood of normotensive and hypertensive dogs were determined. The results are summarized in table 3. The data

are averages of duplicate determinations on 8 normotensive and an equal number of hypertensive animals. It is evident from table 3 that there is no significant difference between the findings in normal or hypertensive animals.

DISCUSSION. The results of the above-described studies on the dog indicate that this species reacts qualitatively like the rat (4, 12, 15) and the rabbit (10, 17) to various operative procedures and that the conclusions drawn on the basis of experiments on the latter animals are applicable to the former and presumably also to the human. Chronic hypertension in the dog is not the result of the liberation by an ischemic or otherwise injured kidney of a pressor substance into the circulation since removal of the constricted kidney fails to abolish it and the blood pressure is maintained even in the absence of all renal tissue. The contrary conclusions of previous investigators must be attributed to the fact that they were based on the abolition of the elevation in blood pressure within a few days or at most a few weeks following the constriction of a renal artery (1, 2, 5, 23). This procedure induces the production of a pressor substance (presumably renin, angiotonin or hypertensin, as it has been variously designated), but this is only a temporary reaction and is in no way related to the permanent chronic hypertension which is ultimately established (9, 10).

In considering the pathogenesis of chronic experimental hypertension, it is necessary to differentiate between the acute rise due probably to tissue necrosis induced by renal ischemia and the permanent rise which constitutes chronic hypertension. The latter requires a period of weeks for its appearance nor does it require the presence of renal tissue. In fact, as has been emphasized previously (8), it is the absence of functional renal tissue rather than the presence of "ischemic" tissue which apparently determines the presence of hypertension. The failure of bilateral nephrectomy to induce hypertension does not contradict this view since a definite time must elapse before the renal deficiency manifests itself in a rise in blood pressure. If death from renal insufficiency be prevented as for example in the parabiotic animal (14) hypertension follows bilateral nephrectomy.

Ogden and his co-workers (18) have explained their observations on the rat by assuming that the early rise in blood pressure is humoral in origin while the latter rise is nervous in origin and secondary to the former. The latter assumption, however, is unnecessary since chronic hypertension in this species (12) as in the dog can be induced gradually and without any preliminary marked rise soon after operation. Pickering (17), confirming earlier work on the rabbit (10), has also concluded that "a non-renal factor plays an important, and perhaps the chief, rôle in maintaining the raised pressure" observed in this species. He noted the absence of structural changes in the blood vessels to account for this non-renal factor, a conclusion in accord with the findings of Halpert and the author (16). It is unnecessary, however, to assume that the factor involved is "non-renal" if one assumes that the kidney normally manifests an incretory or humoral function interference of which by nephrectomy, constriction, ischemia, toxic agents, etc., results in a deficiency which is responsible for the observed rise in blood pressure (8, 9).

The failure of the hypertensive dog to respond to sodium restriction indicates

that this procedure must be considered as only palliative and only effective in such cases as manifest a plethora of the circulation with the abnormal retention of sodium and water in the blood and extra-cellular fluids.

The available data suggest that at least some form of chronic hypertension as it is observed in the human is of renal origin and presumably has the same pathogenesis as in the experimental animal. If this be the case, it is not surprising that nephrectomy only exceptionally results in a lowering of the blood pressure in unilateral renal disease. Why a drop in blood pressure should occur in the exceptional case is not clear. Several possibilities suggest themselves. A contracted non-functioning kidney may perhaps release a pressor substance into the circulation and its removal would thus be comparable to that observed experimentally following removal of a kidney the artery to which had been constricted sufficiently to give a transient rise in blood pressure. It is also possible that the presence of the atrophic kidney, although non-functional, prevents the compensatory reaction of the opposite normal organ.

SUMMARY

The effect of various procedures (constriction, nephrectomy) on the blood pressure of the dog was determined. The results indicate that the immediate rise in pressure which occurs following the constriction of a renal artery is humoral in origin but unrelated to the chronic rise which occurs later and the pathogenesis of which is different. Either constriction or removal of one kidney in the dog, the other remaining intact, results ultimately in a slight but definite elevation in blood pressure. Removal of the constricted kidney does not abolish chronic hypertension which is maintained also for some hours even in the absence of all renal tissue. These findings on the dog confirm earlier experiments on the rat and rabbit. Their bearing on the possible pathogenesis of chronic hypertension is indicated.

The hypertensive dog, like the rat and human, reacts with a fall in blood pressure to the administration of oxidized oils but fails to respond to drastic salt restriction. Studies of the sodium and chloride content and of the carbon dioxide combining power of the blood indicate that these values are normal in the hypertensive dog. This is suggested as the explanation of the failure of response of this species to drastic sodium restriction.

REFERENCES

- (1) BLALOCK, A. AND S. E. LEVY. *Ann. Surg.* **106**: 826, 1937.
- (2) BRAUN-MENENDEZ, E., J. C. FASCILOLO, L. F. LELOIR, J. M. MUNOZ AND C. TAQUINI. *Hypertension Arterial Nefrogena. Libereria y Editorial El Ateneo, Buenos Aires*, 1943.
- (3) BUTLER, A. M. AND E. TUTHILL. *J. Biol. Chem.* **93**: 171, 1931.
- (4) FRIEDMAN, B., J. JARMAN AND P. KLEMPERER. *Am. J. Med. Sci.* **202**: 20, 1941.
- (5) GOLDBLATT, H. *Ann. Int. Med.* **38**: 11, 69, 1937.
- (6) GROLLMAN, A. *Proc. Soc. Exper. Biol. and Med.* **57**: 102, 1944.
- (7) GROLLMAN, A. *J. Pharmacol. and Exper. Therap.* **84**: 128, 1945.
- (8) GROLLMAN, A. *Essentials of endocrinology*. 2nd ed. J. B. Lippincott Co., Philadelphia, 1946.

- (9) GROLLMAN, A. Special Publications, New York Acad. Sci., **3**: 99, 1946.
- (10) GROLLMAN, A. This Journal **142**: 666, 1944.
- (11) GROLLMAN, A. AND T. R. HARRISON. Proc. Soc. Exper. Biol. and Med. **60**: 52, 1945.
- (12) GROLLMAN, A., T. R. HARRISON AND J. R. WILLIAMS. This Journal **139**: 293, 1943.
- (13) GROLLMAN, A., T. R. HARRISON, M. F. MASON, J. BAXTER, J. CRAMPTON AND F. REICHSMAN. J. A. M. A. **129**: 533, 1945.
- (14) GROLLMAN, A. AND C. RULE. This Journal **138**: 587, 1943.
- (15) GUADINO, N. M. Rev. Soc. Argent. biol. **20**: 546, 1944.
- (16) HALPERT, B. AND A. GROLLMAN. Proc. Soc. Exper. Biol. and Med., **62**: 273, 1946.
- (17) PICKERING, G. W. Clin. Science **5**: 229, 1945.
- (18) REED, R. K., L. A. SAPIRSTEIN, F. D. SOUTHARD AND E. OGDEN. This Journal **141**: 707, 1944.
- (19) ROBBARD, S. AND L. N. KATZ. Am. J. Med. Sci. **198**: 602, 1939.
- (20) WILLIAMS, J. R., A. GROLLMAN AND T. R. HARRISON. This Journal **130**: 496, 1940.
- (21) VAN SLYKE, D. D. J. Biol. Chem. **58**: 523, 1924.
- (22) VAN SLYKE, D. D. AND W. C. STADIE. J. Biol. Chem. **49**: 369, 1921.
- (23) VERNEY, E. B. AND M. VOGT. Quart. J. Exper. Med. **28**: 253, 1938.

THE FUNCTION OF THE ABDOMINAL CHEMORECEPTORS OF THE RAT AND MOUSE¹

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Goormaghtigh, in 1936, reported the presence of non-chromaffin epithelioid bodies in the abdomen of the mouse. These bodies are situated especially in the region of the right vagus nerve and the gastric artery, between the diaphragm and the coeliac plexus; Goormaghtigh believed them to be innervated by motor fibers of the vagus and called them "abdominal vagal paraganglia." However, anatomical studies (Hollinshead, 1941) have indicated that the nerve supply to these bodies is sensory, for the innervating fibers appeared to be derived from the dorsal root ganglia of spinal nerves. Because of this, and the fact that these cell groups are morphologically indistinguishable from the carotid body, I have regarded them as abdominal chemoreceptors. Similar abdominal bodies occur in the rat, though more sparsely than in the mouse, and there has been presented (Hollinshead, *op. cit.*) some slight evidence that their stimulation may accelerate respiration in both these forms.

There is abundant physiological evidence that in most animals there are no chemoreceptors other than those (carotid bodies, aortic bodies) innervated by the ninth and tenth cranial nerves which are capable of influencing reflexly either respiration or systemic blood pressure. However, this does not invalidate the interpretation that the abdominal epithelioid bodies of the mouse and rat are chemoreceptors. A careful search for non-chromaffin epithelioid bodies along the abdominal vagi of the cat, guinea pig, and rabbit has revealed none. Thus the available morphological and physiological evidence indicates that the mouse and rat are exceptions in their possession of such bodies.

While there is strong anatomical evidence that the abdominal epithelioid bodies in these two forms are chemoreceptors, their possible effect upon blood pressure has apparently never been investigated, and the evidence that their stimulation may affect respiration is not conclusive. The work reported here is an attempt to substantiate the occurrence of chemoreceptor reflexes initiated in the abdominal area, and to localize their origin in the abdominal bodies. The results indicate that while the respiratory effect of stimulation of the abdominal epithelioid bodies is slight, their effect upon blood pressure, at least in the rat, is quite marked.

MATERIAL AND METHODS. The greater part of this work, including all of the measurements of blood pressure, was carried out upon the rat. Although, according to previous investigations, the abdominal bodies of the mouse are more constant and better developed than those of the rat, investigations of this type on the former animal present many difficulties because of its size.

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Respiratory movements in the mouse were recorded from the thoracic cage, and in the rat from either this or the diaphragm. Blood pressure in the rat was recorded by a mercury manometer attached to a cannula in the abdominal aorta. The tube from the cannula was filled with citrate and heparin. The experiments were performed under light nembutal anesthesia.

Removal of the carotid bodies in the mouse can usually be effected through extirpation of the superior cervical ganglia, as the carotid bodies in this animal are closely attached to, or even imbedded in, these ganglia. In the rat it was found necessary to remove also the carotid bifurcation itself. The removal of the carotid bodies in both species was confirmed by subsequent histological examination. The chemoreceptor tissue usually found about the heart (supra-cardial bodies, aortic bodies²) is rather poorly developed in the rat, and a careful search has revealed no similar tissue in this location in the mouse, but, as an

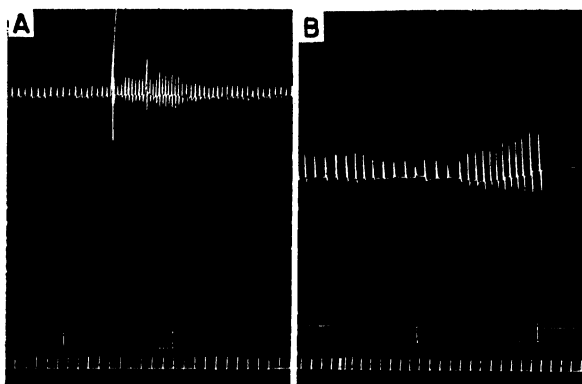


Fig. 1. Respiratory responses to inhalation of nitrogen in the mouse. A shows the response of an animal with intact chemoreceptors, B the less pronounced but still distinct response obtained in a typical case after vagotomy and removal of the carotid bodies. From above downward are shown: respiration, signal, time in seconds.

added precaution, possible chemoreceptor effects from the cardiac region were eliminated by section of the cervical vagi.

EXPERIMENTS. The experiments fall into two general series: in the first, the effect of the chemoreceptor tissue in the neck and thorax was eliminated as described above, and the effects of inhalation of nitrogen or intravenous injection of cyanide were recorded. In the second series, a small amount of cyanide solution was applied directly to the region of the abdominal bodies, either from a syringe or by a small cotton applicator. Both of these were of value in indicating the origin of chemoreceptor reflexes from the abdominal bodies.

The respiratory effects of nitrogen inhalation in the mouse are shown in figure 1, A and B, and the effects of intravenous cyanide are similar. The response of the normal mouse to acute anoxia induced by either of these methods

² For other synonyms, of which there are many, see Hollinshead, 1940, p. 37.

is much more marked than that occurring after extirpation of the carotid bodies and section of the cervical vagi, and is characterized by both increased amplitude and speed of the respiratory movements. After elimination of the carotid bodies, and with the deeper respiration produced by section of the vagi, the effect may be limited to an increase in respiratory rate; it has, however, been observed in every case in this small series (12), and gives evidence for the existence of chemoreceptors other than those in neck and thorax. Attempts at localizing the chemoreceptor effect more exactly in the mouse have been largely unsuccessful because of technical difficulties. However, the involvement of the abdominal epithelioid bodies in this reflex activity is indicated by the fact that three animals which survived the additional operation of bilateral section of the splanchnic nerves and of the abdominal vagi failed to show further respiratory responses to cyanide.

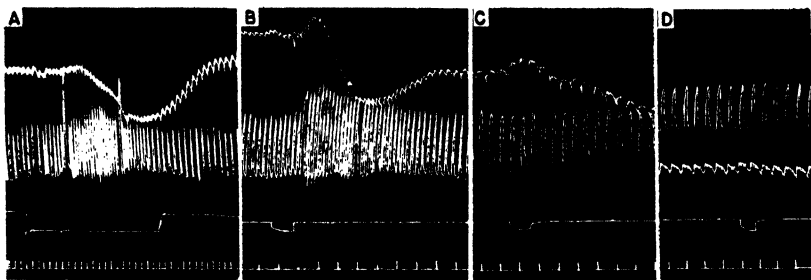


Fig. 2. Vasomotor and respiratory reflexes in the rat. A, the effects of nitrogen inhalation in the normal animal; B, C, effects of intravenous cyanide in an animal with (B) all chemoreceptors intact, and (C) carotid and aortic bodies eliminated; D, non-effect of cyanide in the same animal after denervation of the abdominal bodies. From above downward, in A, B, and C, are shown blood pressure, respiration, the signal, and time. In D, respiration is recorded above blood pressure. A, time in seconds, B-D time in 3-second intervals.

The responses obtained from the rat were somewhat more variable. Under the conditions of these experiments, the normal rat responded to nitrogen inhalation with increased respiratory movements but a fall in blood pressure (fig. 2, A). Extirpation of the carotid bodies and section of the vagi did not, of course, change the vascular response to nitrogen, and the use of nitrogen inhalation as a chemoreceptor stimulant was therefore abandoned; however, the effect of nitrogen upon respiration, following elimination of cervical and thoracic chemoreceptors, was tested in seven animals. Only five of these showed any respiratory stimulation, and this was much less marked than in the mouse.

Intravenous cyanide (0.05 cc. of 0.1 per cent KCN) in the normal rat produced both a marked increase in respiratory movements and a rise in blood pressure of 5-18 mm. (fig. 2, B). After elimination of the carotid and aortic chemoreceptors, respiratory effects of cyanide were largely abolished, as in the similar experiment with nitrogen, and the slight respiratory stimulation sometimes

obtained was not at all constant. In six of these animals, intravenous cyanide produced increases in blood pressure of 4–14 mm. (fig. 2, C); in the other two cases, neither respiratory nor vascular reflexes were produced. In those operated animals in which intravenous cyanide had effected an increase in blood pressure, section of both abdominal vagi and both splanchnic nerves completely eliminated (fig. 2, D) the response previously obtained from cyanide.

As the above experiments indicated that vascular responses of apparent chemoreceptor origin can be elicited in the majority of rats after elimination

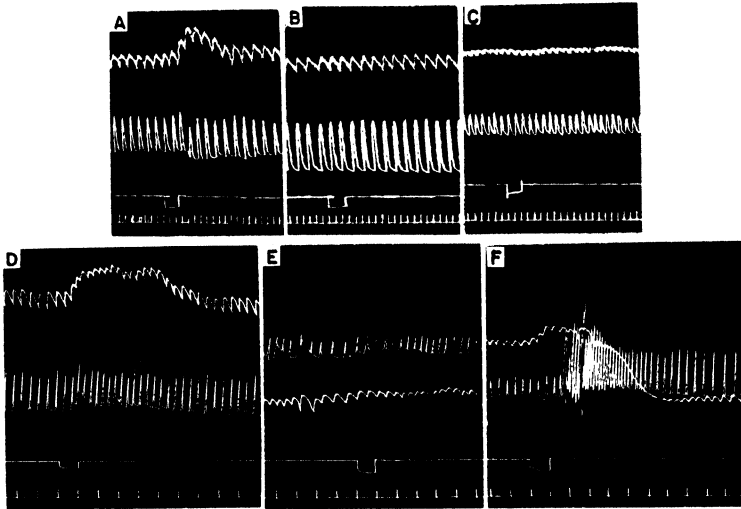


Fig. 3. A, B, C are recordings from one animal showing (A) the vascular response to cyanide applied locally to the abdominal epithelioid bodies, (B) the lack of response from a similar application of cyanide to the small intestine, and (C) the loss of the response to locally applied cyanide following denervation of the abdominal bodies. D, E, F are tracings from another animal showing (D) the vascular (and slight respiratory) effect of cyanide applied locally to the abdominal bodies, (E) the absence of this effect following denervation of these bodies, and (F) reflexes of aortic and carotid body origin produced by intravenous cyanide after denervation of the abdominal bodies. From above downward are blood pressure, respiration, signal, and time, except in E, where blood pressure was recorded below respiration. A-C, time in seconds, D-F, time in 3-second intervals.

of the cervical and such thoracic chemoreceptors as may be present, but not after the additional denervation of the abdominal area, further experiments to localize the origin of the vascular response were carried out. In animals with carotid bodies and cervical vagi intact, local application of cyanide to the peritoneum over the region of the abdominal bodies produced as a rule a fairly prompt rise in blood pressure (fig. 3, A and D), and sometimes a very slight respiratory stimulation. The rise in blood pressure is not so abrupt as after intravenous injection of cyanide, probably because the cyanide must diffuse into the abdominal bodies rather than reach them through the blood stream, but the amplitude of the rise compares very favorably with that obtained with

vascular injections, ranging between 4 and 20 mm. Applications of similar amounts of cyanide to the gut or other regions of the peritoneum have produced no effect (fig. 3, B), though larger amounts of cyanide in such locations have given both vascular and respiratory responses. These may be distinguished from the effects of local application to the abdominal bodies by the prolonged time (approximately 15 or more seconds) intervening between the time of application and the response.

The effects of cyanide applied to the abdominal bodies have invariably been abolished (fig. 3, C and E) by section of the splanchnic nerves and of the abdominal vagi. After such section intravenous cyanide, which of course reaches the carotid body, will still produce both respiratory and vascular effects (fig. 3, F).

DISCUSSION. The effects of nitrogen inhalation and of intravenous cyanide in both the mouse and rat, after removal of the carotid bodies and denervation of possible chemoreceptors about the heart, apparently indicate the existence of chemoreceptors in other regions of the body. The disappearance of these responses after section of the splanchnic nerves and abdominal vagi tends to show that they cannot be due to direct stimulation of the respiratory or cardiovascular centers, but have their origin in chemoreceptors located in the abdomen.

According to previous findings, the abdominal epithelioid bodies are numerous in the mouse, but may be poorly represented in the rat. In line with this is the regular occurrence of reflexes arising from the abdominal area in the former and the less regular occurrence of such reflexes in the latter.

The more exact localization of the origin of chemoreceptor reflexes in the abdomen, as reported here in the rat, seems to prove that the abdominal epithelioid bodies are in fact the chemoreceptors concerned. If we confine our attention for the moment to the vascular responses exclusively, since the respiratory responses in the rat seem hardly trustworthy, the truth of the above statement should be apparent. The rather prompt response obtained from application of cyanide to the region of the abdominal bodies and the absence of such response, or even the occasional delayed response, obtained when cyanide is applied to other regions of the peritoneum, indicate that the rise in blood pressure in the first instance is due to stimulation of some structure or structures within the relatively localized field—that is, in the mesenteric area between esophageal hiatus and coeliac ganglion. That the observed rise in blood pressure is not produced by direct stimulation of the coeliac ganglion is shown by the fact that similar application of cyanide has produced no vascular response after section of the splanchnic nerves (and abdominal vagi), although care was taken in this operation not to injure the coeliac ganglion or its post-ganglionic fibers. Finally, the absence of a response from local application of cyanide to the denervated abdominal region cannot be due solely to destruction of the effector side of the vasopressor reflex arc; intravenous injections have shown that the carotid bodies, which use the same effector system, may still exert a vasopressor effect after that from the abdomen has disappeared. This disappearance of the response from the abdomen, therefore, must be due in part to section of sensory fibers from chemoreceptors in this area.

From anatomical studies¹ of the abdominal epithelioid bodies in the mouse it was reported (Hollinshead, 1941) that the abdominal vagi and the splanchnic nerves both carried dorsal root fibers ending in those bodies. While the number of such fibers through each of these pathways obviously varied from animal to animal, it was felt that, on the average, the abdominal chemoreceptors received about half of their innervation from each. In the present experiments the effects of abdominal vagal section alone, or of splanchnic section alone, upon the abdominal vasopressor response in the rat have been tested in only a few cases. However, the results indicate that the sensory fibers running in the splanchnics are of more importance for the integrity of the reflex than are those in the vagi. Section of the splanchnic nerves alone has greatly reduced or completely abolished the reflex, while vagal section has left it either apparently unaffected, or only somewhat diminished.

It is apparent from the experiments reported here that the abdominal chemoreceptors of the mouse and rat must play, at the most, a minor rôle in the hyperpnea produced in the intact animal by anoxia. The respiratory response obtained from abdominal chemoreceptor stimulation in the mouse is apparently definite, but by no means comparable to that obtained in the intact animal; that from the rat is much less marked, and has frequently not been observed. As the mouse apparently has no chemoreceptors about the heart or the great vessels at the base of the neck, the carotid body of this animal must be responsible for the greater part of the hyperpnea. According to anatomical studies chemoreceptors are present about the heart in the rat, though rather sparse; these may therefore participate in the production of hyperpnea, though it seems probable, both from their poor development and from Comroe's (1939) studies on the dog and cat, that the carotid body is the source of most of the respiratory stimulation.

On the other hand, the abdominal chemoreceptors, at least in the rat, certainly play a part in the production of the vasopressor response to anoxia. The relative participation of the carotid body and of the aortic chemoreceptors in this response cannot be assessed from these experiments, nor is their total contribution to this clear; while presumed stimulation of both of these with the abdominal chemoreceptors denervated has given rises in blood pressure distinctly less than those obtained in the intact animal, this may be due in part to section of the splanchnic nerves. Stimulation of the abdominal chemoreceptors alone, however, has given vasopressor effects sometimes equalling that observed from stimulation of all the chemoreceptors, and averaging somewhat more than half of this.³ The abdominal chemoreceptors of the rat, therefore, and probably, then, those of the mouse, are primarily concerned with the circulatory system rather than with the respiratory system.

Comroe's (op. cit.) beautiful analysis of the functions of the aortic bodies

³ The average rise in blood pressure obtained by intravenous cyanide in rats with all chemoreceptors intact was 10.8 mm. That obtained with intravenous cyanide after elimination of the carotid and aortic bodies was 6.5 mm., while that obtained from local stimulation of the abdominal chemoreceptors was 9.7 mm.

in the dog and cat has indicated that in the former species especially the aortic bodies exert their chief effect upon the vascular system, while the carotid bodies affect especially the respiratory system. It seems probable, therefore, that in the mouse and rat the abdominal chemoreceptors represent the functional equivalent of the aortic bodies of other species.

SUMMARY

Nitrogen inhalation or intravenous cyanide has produced slight respiratory reflexes in each of twelve mice in which chemoreceptors in the neck and thorax had been eliminated. In the rat with known chemoreceptors eliminated, the respiratory response has been much more variable, but with cyanide a clear-cut vascular response was usually obtained. The responses following intravenous cyanide disappeared after section of the abdominal vagi and the splanchnic nerves, which previously have been shown to be the pathways through which sensory fibers reach the abdominal epithelioid bodies of the mouse and rat. Further localization of the origin of a chemoreceptor response from structures within the abdomen was obtained by local application of cyanide to the abdominal bodies; the reflexes so obtained were predominant vasopressor and disappeared following combined abdominal vagal and splanchnic nerve section. The abdominal epithelioid bodies of the mouse and rat appear, therefore, to be chemoreceptors influencing primarily, at least in the rat, the cardiovascular system.

REFERENCES

- COMROE, J. H., JR. This Journal **127**: 176, 1939.
GOORMAGHTIGH, N. J. Anat. **71**: 77, 1936.
HOLLINSHEAD, W. H. J. Comp. Neurol. **73**: 37, 1940; Ibid., **74**: 269, 1941.

CIRCULATORY AND RESPIRATORY REFLEXES IN THE RABBIT DURING TIPPING

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It has been shown that rabbits, when tipped to the head-up position, exhibit a considerable degree of circulatory compensation after loss of the buffer nerves (J. Koch, 1935), and even after splanchnicectomy (Simister and Conklin, 1943). The rise in blood pressure during the tipping period was noticed to be closely related to the integrity of respiration. It was decided for this reason to make a study of respiration during tipping, and its relation to circulatory changes.

METHOD. Forty-two rabbits, of an average weight of 2.45 kgm., were anesthetized intraperitoneally with urethane (1 gram per kgm. body weight, with small additions as necessary) which was used to avoid the depressing effect of the barbiturates on the compensatory reflexes (Bouckaert and Heymans, 1937). Ether was necessary during splanchnic isolation, but was immediately discontinued when that was accomplished. The vagi and aortic nerves were isolated in the neck, the left carotid artery was cannulated for blood pressure recording and a lifting ligature laid under the right carotid artery. Lifting ligatures were also laid under the splanchnic nerves, which were isolated by the retroperitoneal approach. The later sectioning of all nerves was carefully checked at autopsy, and no experiments were included in which this was incomplete.

Blood pressure was recorded kymographically by means of a metal bellows manometer, calibrated against a mercury manometer. Respiration was recorded graphically by means of a Silverman (1946) recorder, the prime mover of which consists essentially of a 400-mesh monel metal screen mounted and attached during experiments to the tracheal cannula. This screen is designed to give the minimum of resistance needed to operate the instrument. Behind the screen are a manifold and a small tube connecting with the recording device. The pressure drop across the screen, as the air passes to and from the animal's lungs, is a linear function of the air flow. A dry cell is connected across the screen, whose resistance warms the air and so prevents condensation of moisture from expired air, which would influence the calibration.

The recording device, connected by tubing with the prime mover, consists of a sensitive wafer bellows and linkage actuating a small mirror, which reflects a beam of light from a slit on to the moving paper of a camera, so that a continuous record of respiration can be taken. The whole apparatus is calibrated with a flow meter so that measurements of the records yield figures on practically any desired aspect of breathing.

For each experiment records of respiration were taken with a face mask before and after anesthetization. After a tracheal cannula had been inserted, the prime mover was attached to the cannula. Respiration records were taken with the rabbit in the horizontal position, before and after each experimental procedure, and in the tipped position during each tip. Continuous blood-pressure records were made before, during, and after each tip, and these records were measured for changes in blood pressure and pulse rate.

The experimental procedure was to tip the animal to 60°, head up, for about 35 seconds. Sixty degree tips, while producing a response qualitatively the same as those at a greater angle, are less hard on the animal; and thirty-five seconds was chosen because it was long enough to include the compensatory response. This was done first after the dissection was completed, and constituted a "normal" tip. The tipping was repeated three times, after progressive elimination of pressoreceptors in the following order: 1. Sectioning of vagi and aortic nerves; although the rabbit has separate aortic nerves, some depressor fibers are carried in the vagi (Boyd and McCullagh, 1938). 2. Temporary clamping of the right carotid artery, which, together with the cannulation of the left carotid artery, functionally eliminated the carotid receptors. This procedure was followed in preference to sinus denervation to minimize trauma. Witt, Katz and Kohn (1934) warn that careful denervation of the carotid area often causes death from respiratory failure. Since discharges over the sinus nerve in rabbits are abolished by a reduction of sinus pressure to 50 mm. Hg (Bronk and Stella, 1932), functional elimination is justifiable. 3. Section of the splanchnic nerves. A recovery interval of at least five minutes was allowed between each two tippings. In a few cases curare was given after section of the splanchnic nerves, so that the effect of lack of skeletal muscle tone, especially that of the respiratory muscles, could be tested.

RESULTS AND DISCUSSION. When a rabbit is tipped, head up, the hydrostatic effect causes an immediate drop in blood pressure and some decrease in respiratory minute volume. There is practically always a compensatory rise in blood pressure during the tipped period. A few extremely vigorous animals have shown little or no drop in blood pressure and a compensatory rise above the horizontal level, but this is an unusual response. Following the experimental procedures outlined above there is a general trend downward in compensatory reactions.

Circulation. A summary is given in table 1 of the circulatory changes occurring as a result of experimental tipping and elimination of receptors. These results, which confirm and extend those previously obtained, show insignificant changes in pulse rate of at most ± 6 per cent. Changes in blood pressure in the course of the experiment are much more marked, though the net change from the beginning of the experiment is only -9 per cent.

Clamping the carotid arteries and section of the aortic nerves both remove depressor influences, but vagotomy also abolishes pressor influences from the great veins and the right heart. The direction of change in the blood pressure of any animal will reflect the balance of these factors. The splanchnic nerves

not only contain constrictor fibers but also afferent fibers arising in the Pacinian corpuscles, which signal the degree of distention of the mesenteric vessels (Gammon and Bronk, 1935). Proof that the mesenteric vessels participate in blood pressure regulation was given by Heymans *et al.* (1930b, 1936).

In absolute terms, compensation during tipping increases with each of the two steps in the elimination of the carotico-aortic mechanism. When calculated as percentage, the only marked change occurs with the sectioning of the vagi and aortic nerves, when compensation is notably increased. In the four control experiments in which the clamp was not put on the right carotid artery, so that the pressoreceptors on one side were functional, though vagi, aortics, and splanchnics had been sectioned, the compensation was only 9.8 per cent, as compared with 18.5 per cent when the carotids were both functionally eliminated. It is clear the ability to compensate is not easily destroyed, and that it is actually enhanced by removal of the carotico-aortic reflexes.

TABLE 1

Summary of circulatory changes during tipping experiments (means)

PROCEDURE	NO. OF EXPTS	PULSE RATE		BLOOD PRESSURE							LENGTH OF TIP
		Initial	During tip	Initial	During tip						
					Low-est	Time	High-est	Time	Compensation		
									mm. Hg	sec.	
		per min.	per min.	mm. Hg	mm. Hg	sec.	mm. Hg	sec.	mm. Hg	%	sec.
Normal.....	20	290	275	161	90	9	118	27	28	17.4	34
Vagi and aortics cut (VA).....	8	275	274	174	130	7	169	31	39	22.4	36
VA and carotid clip (VAC)....	14	288	305	219	165	9	206	27	41	18.7	36
VAC and splanchnics cut (VACS).....	8	291	292	146	86	17	113	34	27	18.5	40
VAS controls.....	4	275	273	156	107	10	120	29	13	9.8	35

These results agree with those of J. Koch (1935) for rabbits, but are at variance with Edholm's (1940) results on cats, whose recovery in the tipped position was not affected by removal of carotid sinus impulses, but was decreased by vagotomy. Mayerson (1942) found in dogs that denervation of the carotid sinuses or cutting the vagi diminishes the animal's ability to compensate for gravity. Apparently there is a species difference. Edholm (1942) has proved that when cats are tipped to the head-up position the fall of blood pressure is largely due to accumulation of blood in the liver, but that splanchnic constriction tends to compensate for the fall. In view of the fact that rabbits show a fair degree of compensation when even the splanchnic nerves have been sectioned, one must search for other pressoreceptors or look for compensation by means of skeletal muscle tension and the movements of respiration.

Compensation was entirely lacking when the rabbit was given curare and then tipped after respiration had practically ceased. Figure 1 shows the change from the perceptible compensation occurring even after loss of vagi, carotico-aortic mechanism, and splanchnic nerves, to the steady decrease in blood pressure

during tipping after curare. A great relaxation of the abdominal wall was apparent, as well as the absence of respiratory aid to venous return, which made it impossible for the animal, already deprived of the usual reflex mechanisms, to improve its blood pressure when in the head-up position.

Respiration. A series of respiration records for one experiment is seen in figure 2, which is typical, except for the unusually slow rate in the unanesthetized animal. Of these records the entire curve above the base line represents in-

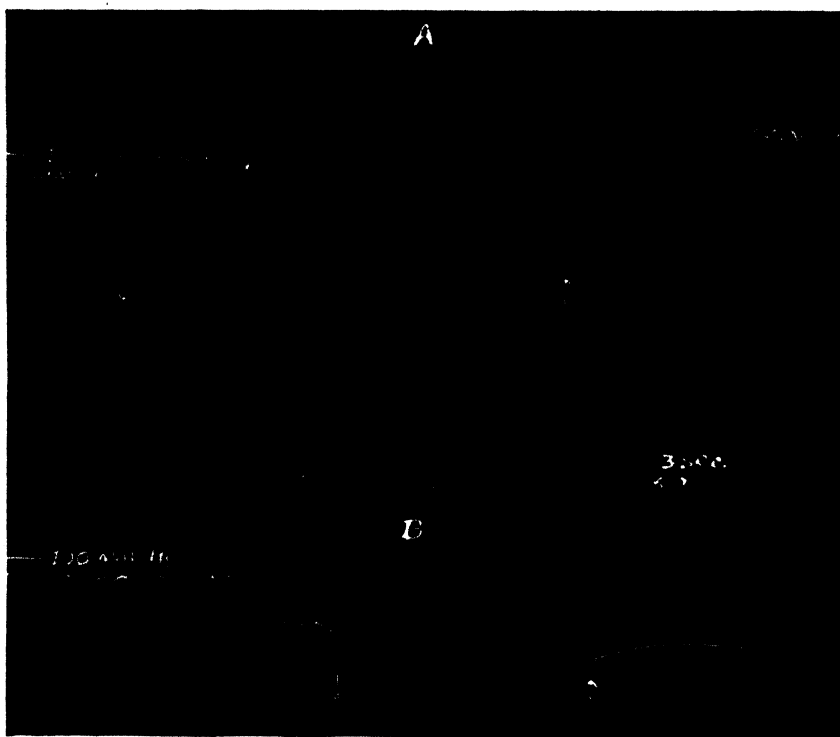


Fig. 1. Effect of curare on blood pressure during tipping. A. Rabbit 39 tipped after elimination of vagi, aortics, carotids, and splanchnics. B. The same rabbit tipped 20 minutes later, after curare.

spiration, that below the base line, expiration. After establishing the identity of minute volume in expiration and inspiration on a few curves, only inspiration was measured on succeeding curves.

A summary of measurements of inspiration for all experiments is contained in table 2. The main points to be noted are as follows:

- a. There is a fairly regular decrease in rate with the progress of the experiment, a significant increase occurring only when both carotid arteries are eliminated.

- b. The percentage of the total cycle occupied by inspiration increases with loss of carotids and vagi.

c. The tidal volume increases more than five-fold with elimination of reflexogenic sources.

d. Mean and maximum flow and minute volume are all gradually reduced by experimental procedures and especially by tipping, but increased by the anoxia resulting from carotid elimination.



Fig. 2. Respiration records, rabbit 30. Curves of inspiration above base line; expiration below. Read from right to left.

e. The ratio of maximum flow to minute volume shows a gradual tendency to increase with experimental procedures, and especially with tipping, when the minute volume is always lowered.

In this series of experiments we are dealing with two sets of experimental changes, the reduction of carotid and aortic chemoreceptors and the postural effect on respiration. To these must also be added the depressant effect of the anesthetic, which is much less marked with urethane than with ether or the barbiturates. Gordh (1945), with deep barbiturate anesthesia, obtained complete respiratory arrest in the tipped position in rabbits. In the experiments

here reported complete cessation of respiration in the head-up position was never seen unless the animal was moribund. This more favorable situation was probably due to the comparative lightness of the anesthesia, and to the use of urethane, shown by Marshall and Rosenfeld (1936) to be less likely to produce respiratory depression than barbiturates, even when deep anesthesia is used.

Temporary elimination of carotid reflexes through occlusion of the common carotid produces hyperpnea, as shown by Heymans and Bouckaert (1930a), and increased minute volume. Section of the aortic nerves also produces a large increase in minute volume, but has little effect on rate. Even a portion of an intact aortic nerve holds down the minute volume. This nerve is extremely fine, and, occasionally, double. In four experiments in which one nerve or a

TABLE 2
Summary of respiratory changes (means)

PROCEDURE	NO. OF EXPTS	RATE	INSP. PHASE % OF TOTAL	TIDAL VOL.	MEAN FLOW	MAX. FLOW	MINUTE VOL.	MAX. FLOW MIN VOL.
				ml.	L./min.	L./min.	L.	
1. Normal.....	28	201	46.1	5.6	2.16	3.42	1.00	3.7
2. Anesthetized.....	26	163	44.8	6.2	1.78	2.83	0.80	3.7
3. Dissection completed.....	18	165	42.2	5.6	1.72	2.78	0.77	3.7
4. Carotid cannula in left carotid.....	12	90	44.8	10.0	1.66	2.63	0.74	3.6
5. Right carotid temporarily clamped..	12	135	48.0	8.5	2.03	3.24	0.97	3.4
6. Normal tipped.....	20	54	38.0	9.8	1.45	2.46	0.51	5.1
7. Aortics only cut.....	5	62	47.4	16.1	1.97	3.10	0.93	3.3
8. Vagi only cut.....	4	43	53.7	23.2	1.80	3.32	0.99	3.7
9. Vagi and aortics cut.....	7	33	42.2	24.5	1.91	3.37	0.81	4.3
10. V-A tipped.....	7	32	43.4	15.0	1.14	2.79	0.48	4.9
11. V-A plus both carotids out.....	10	33	42.0	28.2	2.22	4.02	0.88	5.0
12. V-A-C tipped.....	13	39	38.5	12.0	1.09	2.34	0.44	6.1
13. V-A-C plus splanchnics.....	5	35	44.2	28.8	2.34	3.98	1.01	4.0
14. V-A-C-S tipped.....	5	26	37.0	13.6	1.04	2.26	0.37	7.0

part of a nerve was found at autopsy to have been missed, the mean minute volume was 19 per cent less than when aortic section was complete. Vagotomy also increases minute volume; a mean gain of 34 per cent may be seen if a comparison is made between procedures 4 and 8 in table 2. This greater ventilation reduces anoxia and helps mechanically to increase the compensatory rise in blood pressure during tipping. However, a gradual decline in ability to meet an emergency situation, such as the head-up posture, is shown in figure 3 by the increasing loss in minute volume with tipping under different conditions. Although splanchnicectomy increases minute volume, this is probably explained by reduced cerebral blood flow and accumulation of CO₂ in the respiratory center. Anoxia, while undoubtedly present, would have little effect on the center other than to depress it, and would be unable, under conditions of the experiment, to act upon the carotid and aortic bodies.

The mechanical effect of tipping to a 60° angle obviously interferes with the respiratory pump action. Although Eyster and Hicks (1932) stated that in dogs the effect of breathing on venous return is not as great as ordinarily believed, Woodbury and Abreu (1944) showed in dogs, using only local anesthesia for insertion of cardiac sounds and intrathoracic and intra-abdominal balloons, that normal respiration, and to a greater extent deep breathing, does increase venous return to the heart and produce a more effective systole. Dying gasps even pumped blood into the lungs, brain, and coronary circulation after cardiac arrest.

This respiratory pump action is, in fact, practically the only agency left to aid venous return from the hind limbs and splanchnic region under procedure

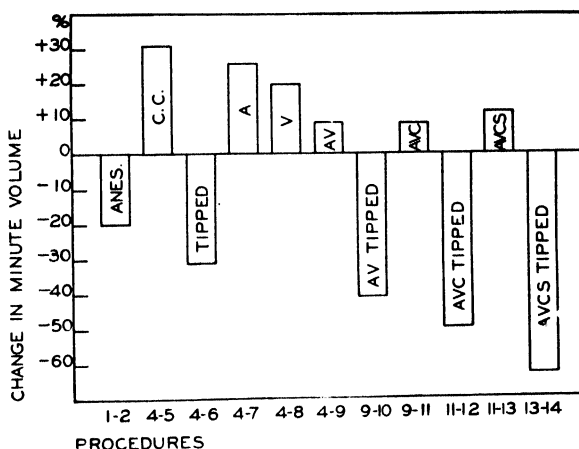


Fig. 3. Cumulative effect of experimental procedures on minute volume. CC: carotid arteries clamped; A: aortic nerves cut; V: vagi cut; AV: aortics and vagi cut; AVC: aortics and vagi cut, carotids clamped; S: splanchnics cut. Numbers on abscissa refer to table 2.

14 (table 2). The fact that blood pressure still shows compensation under these conditions indicates the importance of this mechanism. The complete disappearance of compensation when curare is given is further proof of the value of the respiratory pump to the animal under severe strain.

SUMMARY

Rabbits, anesthetized with urethane, were subjected to 60° tipping to the head-up position for about 35 seconds, before and after progressive elimination of receptors which normally initiate reflex control of circulation and respiration. Blood pressure and respiration were recorded and measured and the results are summarized as follows:

1. The pulse rate remains practically unchanged.
2. Blood pressure drops when the animal is tipped, but a compensatory rise occurs during the tipping period. This rise is increased by section of the vagi

and aortic nerves. It persists even after splanchnicectomy, but is entirely lost if curare is given.

3. Respiratory rate is increased by functional elimination of the carotids, but shows a general tendency, especially marked after vagotomy, to decrease in the course of the experiment.

4. In general, the ventilation is increased by the elimination of the carotico-aortic mechanism and by splanchnicectomy, but is progressively decreased by successive tipplings.

5. After the loss of the carotid and aortic receptors and the splanchnic nerves, the respiratory pump is the chief means of venous return when the animal is tipped to the head-up position.

The writer gratefully acknowledges the hospitality of Dr. Cecil K. Drinker's laboratory and Dr. Leslie Silverman's instruction and help in the use of the respiration recorder.

REFERENCES

- BOUCKAERT, J. J. AND C. HEYMANS. *J. Physiol.* **90**: 59P, 1937.
BOYD, J. D. AND G. P. McCULLAGH. *Quart. J. Exper. Physiol.* **27**: 293, 1938.
BRONK, D. W. AND G. STELLA. *J. Cell. and Comp. Physiol.* **1**: 113, 1932.
EDHOLM, O. G. *J. Physiol.* **98**: 79, 1940; *J. Physiol.* **101**: 1, 1942.
EYSTER, J. A. E. AND E. V. HICKS. *This Journal* **101**: 33P, 1932.
GAMMON, G. D. AND D. W. BRONK. *This Journal* **114**: 77, 1935.
GEMMILL, C. L., E. W. OVERSTREET AND L. M. HELLMAN. *This Journal* **104**: 443, 1933.
GORDH, T. *Acta Chirur. Scand.* **92**: Supp. 102, 1945.
HEYMANS, C. AND J. J. BOUCKAERT. *J. Physiol.* **69**: 254, 1930a.
HEYMANS, C., J. J. BOUCKAERT AND L. DAUTREBANDE. *Compt. Rend. Soc. Biol. Paris* **105**: 217, 1930b.
HEYMANS, C., J. J. BOUCKAERT, S. FARBER AND F. Y. HSU. *This Journal* **117**: 619, 1936.
KOCH, J. *Ztschr. f. Biol.* **96**: 314, 1935.
MARSHALL, E. K. AND M. ROSENFELD. *J. Pharmacol. and Exper. Therap.* **57**: 437, 1936.
MAYERSON, H. S. *This Journal* **136**: 381, 1942.
SILVERMAN, L. *J. Ind. Hyg.* **28**: 1946 (accepted for publication).
SIMISTER, T. H. AND R. E. CONKLIN. *This Journal* **138**: 391, 1943.
WITT, D. B., L. N. KATZ AND L. KOHN. *This Journal* **107**: 213, 1934.
WOODBURY, R. A. AND B. E. ABREU. *This Journal* **142**: 721, 1944.

ASPHYXIAL DEPOLARISATION IN THE SPINAL CORD

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Furusawa (1929) and Cowan (1934) observed a decrease of the injury potential during asphyxiation of excised crustacean nerves. The same had been found by Koch (1927) in peripheral mammalian nerve during circulatory arrest. Gerard (1930), using mainly frog nerve, found that an asphyxiated part of the nerve becomes negative with respect to an oxygenated portion. It was the experience of all authors that these potential changes are quickly reversible on re-admission of oxygen, and they were considered as the expression of a reversible depolarisation of a normally polarised membrane in the nerve fibre.

It is likely that the various parts of the neuron differ considerably in sensitivity to oxygen lack. Since gray matter has a higher oxygen consumption than white matter (Holmes, 1930; van Harreveld and Tyler, 1942), it is probable that the gray matter elements in the spinal cord, especially the nerve cells, are depolarised more rapidly by lack of oxygen than the axons in roots and white matter. During cord asphyxiation, then, a potential difference can be expected to develop between the depolarised nerve cells and their still polarised axons, and it might be possible to lead off these "depolarisation potentials" from an active electrode in the gray matter and an indifferent one placed on a root or on the white matter of the cord.

The existence of depolarisation potentials during asphyxiation of the cord has been examined in the present investigation, and an attempt has been made to correlate the changes in the polarised structures thus recorded, with the changes in reflex activity during the development of and the recovery from asphyxia.

METHODS. The active electrode consisted of a silver wire, 1.5 cm. long and 0.3 mm. thick, coated with shellac and cut off obliquely at one end. The slanting surface was coated with silver chloride. This electrode was placed by hand in the spinal cord, with the sharp tip in the gray matter of the last lumbar or first sacral segment, preferably in the anterior horn. The electrode was connected by a coiled thin copper wire with the rest of the apparatus, allowing small movements of the animal without disturbing the electrode. The indifferent electrode was usually brought in contact with an anterior root (usually S1 or L7). This electrode (fig. 1) consisted of a silver chloride coated loop of silver wire (A), placed inside a glass tube (B). With the thread (C), the root (D) was pulled into the glass tube through the wire loop. The tube was closed with a stopper (E) to protect the root against drying. One electrode thus contacted a nervous structure which during cord asphyxiation remained oxygenated

by the air in the tube (B). In some experiments a posterior root was brought in contact with this electrode, in others a coated silver wire was rested against the posterior or lateral column. After placing the electrodes the cord was covered with mineral oil.

The electrodes were connected with the direct current amplifier described in the appendix. This amplifier had a high input resistance and showed after a period of equilibration very little drift, not more than 0.5 millivolt, per hour when the input was shorted with a 10,000 ohms resistance. Replacing this resistance by the electrodes immersed in Ringer's solution did not materially change the drift, but introduced in some instances small irregularities. For the recording a coil type galvanometer (Leeds and Northrup, 2420 C) was used, with a period of 3 seconds when critically damped.

A large iron cabinet, electrically heated to about 37°, was used to shield the preparation and to maintain normal body temperature.

In order to mark the location of the gray matter electrode, some silver was deposited electrolytically in the cord after the experiment. This showed as a black precipitate in the toluidine blue stained sections which were prepared of each cord used in this investigation.

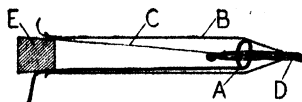


Fig. 1. Electrode, for explanation see text

Cats were used exclusively. Most experiments were performed under light nembutal anesthesia; some decerebrate preparations were examined. To prevent asphyxial convulsions, curare (Intocostrin, E. R. Squibb & Sons, $\frac{1}{2}$ cc/kgm.) was administered in most instances.

Three methods were employed to arrest the oxygen supply of the cord. In the first the apparatus for artificial respiration was stopped, using a curarised preparation; in the second commercial nitrogen was fed into this apparatus. The third consisted in clamping the aorta between the diaphragm and the coeliac artery in preparations in which the cord had been severed at about Th 11 to stop the blood supply through the spinal arteries. The advantage of the latter method is that it can be prolonged without killing the animal, thus making it possible to study the recovery from long periods of asphyxiation. Its disadvantage is that the preparation has to be handled, which sometimes results in electrical artefacts.

As will be discussed later, polarisation of the cord is maintained only when its blood supply is ample. Therefore the blood pressure was recorded continually in most experiments and when it dropped under 8 or 9 cm. of mercury, the pressure was maintained at this level by infusion of adrenalin (10^{-6}) in Ringer's solution.

RESULTS. *Effects of short asphyxiations by stopping the artificial respiration.*

After the artificial respiration of the curarised animals is arrested, a deflection of the galvanometer develops, indicating a negativity of the gray matter with respect to the anterior root. The movement of the galvanometer is slow in the beginning, but soon it speeds up (fig. 2 A). In 20 observations on 15 animals

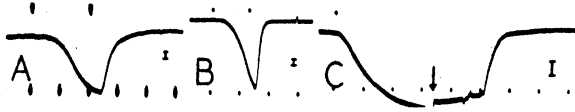


Fig. 2. Depolarisation potentials, A during a 2 minute respiratory arrest; B during nitrogen breathing for 1.5 minutes; C during 5 minutes' clamping of the aorta. In the latter record a small adjustment of the galvanometer position was made at the arrow. In this, as in all following figures, a downward movement of the galvanometer indicates increased negativity of the gray matter. In all three records the deflection for 1 millivolt is shown. Upper signal indicates beginning and end of respiratory arrest, nitrogen breathing or clamping of the aorta; the lower signal is a time signal of 1 minute.

TABLE 1

*Depolarisation during, and repolarisation after a 2 minute respiratory arrest**

NUMBER ANIMAL	START DEPOL.	START REPOL.	COMPLETE REPOL.	POTENTIAL M.V.	REMARKS
1	44"	29"	126"	8.7	Nembutal
2	48"	27"	55"	4.8	Decerebration
3	60"	17"	53"	1.0	Decerebration
4	49"	23"	77"	2.7	Decerebration
5	42"	21"	66"	2.8	Decerebration
6	68"	15"	48"	1.2	Decerebration
7	58"	19"	81"	2.1	Decerebration
8	67"	16"	50"	4.5	Nembutal
9	42"	16"	26"	5.2	Nembutal
10 A	54"	14"	53"	3.8	Nembutal
B	45"	19"	50"	2.8	Nembutal
11 A	49"	15"	44"	3.3	Nembutal
B	49"	18"	40"	3.5	Nembutal
C	43"	24"	57"	4.0	Nembutal
12 A	56"	16"	55"	1.9	Nembutal
B	63"	16"	58"	2.6	Nembutal
C	60"	46"	130"	4.8	Nembutal
13	47"	18"	59"	6.4	Nembutal
14	48"	17"	66"	6.9	Nembutal
15	42"	14"	37"	6.8	Nembutal
Averages.....	52"	20"	62"		

* In all experiments Intocostin was administered.

collected in table 1, the average latent period of this deflection is 52 seconds. The variation of this value may be due partly to differences in the sensitivity of the tissue to oxygen lack, but probably also to the state of the circulation prior to asphyxiation.

The movement of the galvanometer is quickly reversed when after a 2 minute respiratory arrest, the artificial respiration is started again. At first the movement of the galvanometer towards the base line is quick, but then it tapers off and the pre-asphyxial level is reached asymptotically in most instances. Sometimes, however, the base line is crossed and it takes 4 to 5 minutes before the galvanometer is back to that level again. The average latent period of the reversal of the galvanometer movement for the 20 observations of table 1 was 20 seconds. The base line was reached or crossed after an average of 62 seconds. These values show considerable variation, which is probably mainly due to differences in damage of the circulation caused by the two minutes' asphyxiation. In several cases the blood pressure dropped markedly at the end of asphyxiation, and it is obvious that in these instances the re-oxygenation of the cord will be delayed.

Leading off from gray matter and posterior, instead of anterior root, or from gray matter and posterior or lateral column, produced similar depolarisation potentials during 2 minute asphyxiations.

In some experiments non-narcotized decerebrate preparations were used. The depolarisation potentials led off under these circumstances did not differ from those obtained under light nembutal narcosis.

The 2 minute asphyxiations can be repeated with 10 minute intervals many times without consistent changes in the deflections produced.

The size of the potentials recorded during 2 minute asphyxiations differed greatly from experiment to experiment (table 1). No close relation between the electrode placement and the potential size was found; only gross misplacements of the gray matter electrode resulted in absence of the depolarisation potential. It is likely that the mechanical damage and the circulatory disturbance caused by the placement of the electrode in the cord influences the size of the depolarisation potential considerably. The importance of these factors is illustrated by the following observation. For the first 10 to 15 minutes after the placement of the electrodes there is almost invariably a considerable drift of the galvanometer, indicating a decreasing negativity of the gray matter. It is likely that this drift is the expression of the repolarisation of elements depolarised by the disturbance of the electrode placement¹. This viewpoint is supported by the observation that when artificial respiration was stopped for 2 minutes directly after the electrode had been placed in the cord, the resulting galvanometer deflection was very small or absent, but 10 to 15 minutes later, when there was no further drift, asphyxiation produced a sizable deflection.

Effects of stopping artificial respiration permanently. It is obvious from figure 2 A that at the end of a 2 minute asphyxiation depolarisation is not completed. When artificial respiration was stopped permanently, a maximum of negativity of the gray matter was reached relatively quickly; in 12 experiments of this kind (table 2) after an average of 4 minutes. The potential of this

¹ It is of interest to note that Spiegel, Henny, Wycis and Spiegel-Adolf, 1946, found that mechanical disturbances of the brain (concussion) can cause a decrease in polarizability of the cerebrum.

maximum differs widely, between 3.7 and 28.2 millivolts in the same group of experiments. After reaching the maximum the potential usually starts to regress very slowly.

TABLE 2
Final asphyxiation by stopping the respiration

NUMBER ANIMAL	START DEPOL.	MAXIMUM	POTENTIAL OF MAXIMUM M.V.
1	48"	181"	6.6
2	48"	190"	5.2
4	56"	304"	4.0
5	44"	281"	4.2
7	45"	182"	3.7
8	63"	257"	10.9
11	53"	170"	4.0
18	64"	340"	15.5
20	50"	249"	28.2
21	44"	339"	5.8
22	44"	135"	5.9
23	45"	251"	5.0
Averages.....	50"	240"	

TABLE 3
*Depolarisation during, and repolarisation after breathing nitrogen**

NUMBER ANIMAL	DURATION NITRO- GEN BREATHING	START DEPOL.	START REPOL.	COMPLETE REPOL.	POTENTIAL M.V.
10 A	1'30"	19"	16"	49"	1.5
B	1'30"	22"	15"	26"	1.0
11 A	1'	21"	15"	56"	3.7
B	1'	23"	16"	58"	3.8
C	1'	19"	17"	61"	3.9
D	1'	16"	19"	54"	4.2
12 A	1'	22"	20"	88"	4.3
B	1'	27"	23"	78"	3.4
13	1'30"	19"	23"	64"	7.5
15	1'30"	22"	19"	46"	12.6
16	1'	18"	32"	96"	13.0
Averages.....		21"	20"	61"	

* All experiments were performed in Nembutal narcosis, Intocostirin was administered in all instances.

Effects of short periods of anoxia. Feeding nitrogen into the apparatus for artificial respiration also produces negativity of the gray matter with respect to the anterior root (fig. 2 B). The latent period of this deflection, however, is considerably shorter than when the cord is asphyxiated by stopping the artificial respiration. The average latent period of 11 observations on 6 animals was

21 seconds (table 3). Furthermore the deflection develops quicker (fig. 2 A and B). Since breathing nitrogen does not only fail to supply the animal with oxygen, but actually washes oxygen out of the lungs and the blood, the earlier start and quicker development of the depolarisation potential is not surprising.

When after 1 to 1.5 minutes the nitrogen is replaced by air again, the potential regresses in about the same way as after stopping the artificial respiration for 2 minutes. In the experiments of table 3, the recovery started after an average of 20 seconds, and was completed after an average of 61 seconds.

Effects of short asphyxiations by clamping the aorta. Asphyxiation of the cord by clamping the aorta again produces negativity of the gray matter. In table 4 are collected the results of some experiments in which the aorta was clamped for 1 to 5 minutes. The latent period of the deflection is considerably shorter than

TABLE 4
*Depolarisation during, and repolarisation after clamping the aorta for 1 to 5 minutes**

NUMBER ANIMAL	DURATION CLAMPING	START DEPOL.	START REPOL.	COMPLETE REPOL.	POTENTIAL M.V.
17 A	2'	10"	12"	82"	10.2
18 A	1'30"	8"	8"	38"	4.5
13 A	1'	8"	8"	29"	2.4
B	2'	9"	7"	30"	2.5
16 A	1'	7"	8"	85"	5.4
B	2'	7"	8"	35"	5.7
19 A	1'	8"	9"	51"	4.2
15 A	1'	9"	10"	29"	3.3
B	5'	10"	9"	31"	4.7
17 B	5'	8"	7"	79"	2.9
18 B	5'	7"	12"	64"	5.2
19 B	5'	11"	12"	—	5.9
Averages.....		8.5"	9"	50"	

* All experiments were performed under Nembutal narcosis, Intocostin was administered in all cases.

of the effect caused by breathing nitrogen. The average latent period of 12 observations on 8 animals is 8.5 seconds. Clamping the aorta will bring the circulation in the caudal part of the body to a halt almost immediately. Besides the oxygen reserve in gray matter is very small, and will not suffice for more than a few seconds (Roseman, Goodwin and McCulloch, 1946; Bronk, Larrabee and Davies, 1946). These factors account for the very short latent period and the sharpness of inflection (fig. 2 C) of the electrical effect caused by this way of asphyxiation.

In the experiments of table 4 the galvanometer movement is reversed upon removal of the clamp after the very short time of 9 seconds on the average. Since the blood pressure in the anterior part of the body remains high and the blood in the large abdominal vessels does not lose its oxygen during the short asphyxiation, reoxygenation of the cord can start almost immediately after the

release of the clamp, which explains the short latent period of recovery. This period is not markedly longer after 5 than after 1 minute of asphyxiation. The base line is reached on the average after 50 seconds.

Blood pressure and cord polarisation. When the blood pressure drops under 4 to 5 cm. of mercury, the circulation becomes insufficient and a depolarisation potential may develop. The injection of a suitable dose of adrenaline can in such cases cause a temporary repolarisation. Adrenaline has no effect when the blood pressure is reasonably high.

In a few instances the injection of acetylcholine caused a temporary depolarisation, in others similar doses did not do so. It is likely that the depolarisation

TABLE 5

*Depolarisation during, and repolarisation after clamping the aorta for 10 to 30 minutes**

NUMBER ANIMAL	DURATION ASPHYXIATION	DEPOLARISATION			RECOVERY		
		Start depol.	Maximum	Potential of maximum m.V.	Start incr. negat.	Maximum incr. negat.†	Complete recovery
24	10'	12"	181"	24.3	9"	33"	
25	10'	7"	91"	2.9		12"	4'
18	10'	7"	103"	6.3		9"	1'
26	20'	8"	200"	7.6	13"	26"	6'
17	20'	7"	99"	8.5		10"	
27	20'	8"	166"	10.0	19"	31"	
28	20'	11"	90"	6.1	15"	27"	10'
29	30'	11"	189"	31.4	111"	148"	
30	30'	9"	102"	6.6	123"	166"	
31	30'	10"	187"	16.6	27"	57"	
Averages..		9"	141"				

* All experiments were performed under Nembutal narcosis, Intocostrin was administered in all cases.

† When no increased gray matter negativity was recorded, this value indicates the beginning of the galvanometer movement towards the preasphyxial position.

caused in this way is due, not to a specific acetylcholine effect, but to a drop in blood pressure under the critical value.

Effects of asphyxiation in cords with destroyed nerve cells. In 14 cats the spinal cord was asphyxiated for 60 to 70 minutes by forcing Ringer solution in the isolated caudal part of the dural sac under a pressure higher than the blood pressure (van Harreveld and Marmont, 1939). It was found by van Harreveld and Tyler (1942) that this leads in 48 hours to a complete destruction of all the motor cells. Two to 14 days after the 60 to 70 minute asphyxiation the cord was prepared as described above. Two to 2.5 minutes' respiratory arrest or 1 to 1.5 minutes' breathing of nitrogen had no effect in any of the 6 animals examined after 10 to 14 days. In 3 of 8 animals examined after 2 to 3 days, however, these procedures caused a slight negativity of the gray matter which regressed promptly after re-oxygenation of the cord. The highest potential thus observed

was 0.2 millivolt. Sections made of the three cords, in which potentials were elicited by asphyxiation, showed the complete absence of motor cells. The small potentials observed may have been caused by the depolarisation of still intact small nerve cells which were not recognised as such, or of gray matter elements like dendrites (or perhaps bouton terminaux), which might survive the destruction of the perikaryon, till they are destroyed by Wallerian degeneration.

Effects of 10 to 30 minutes' asphyxiations by clamping the aorta. The negativity of the gray matter reached a maximum in 2 minutes 20 seconds on the average after clamping the aorta in the 10 observations collected in table 5. The maximum thus is reached quicker after clamping the aorta than after stopping

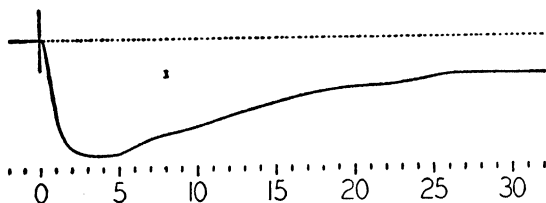


Fig. 3. Depolarisation potential during clamping of the aorta for 40 minutes, redrawn on a convenient scale. The aorta was clamped at the vertical line. Time on the abscissa in minutes. The deflection for 1 millivolt is indicated.

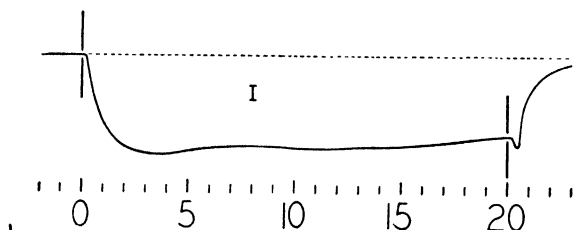


Fig. 4. Potential changes during and after a 20 minute clamping of the aorta. Time in minutes. The aorta is clamped at the first, and released at the second vertical line. Deflection for 1 millivolt is shown.

artificial respiration (table 2). The earlier start and quicker development of the effects of aorta clamping will contribute to this difference. After reaching the maximum, the potential difference usually starts to regress slowly to become stationary after 20 to 30 minutes. In part of the experiments this potential decrease is marked (fig. 3), in others it is less (fig. 4), and in a few instances it is absent.

The recovery after asphyxiations of 10 minutes or longer is often characterized by an initial (relative) increase of the negativity of the gray matter, after which the galvanometer moves towards the pre-asphyxial position (fig. 4). The latter position is often not reached, however, especially after the longer asphyxiations. This is probably an indication of incomplete recovery. Only one of three 10 minute asphyxiations (table 5) showed the initial increase of gray matter nega-

tivity, the others recovered in the same manner as after the 1 to 5 minute asphyxiations. Three of four 20 minute asphyxiations and each of three 30 minute asphyxiations recovered with the initial increase in negativity of the gray matter. Table 5 gives the interval between the release of the aorta clamp and the beginning and the maximum of the initial increase of gray matter negativity. Both intervals become longer with increase of the duration of asphyxiation.

Recovery after 60 and 70 minute asphyxiations. Synaptic conduction has been demonstrated temporarily after 65 minute asphyxiations of the spinal cord (van Harreveld, 1943, 1944b). Sections prepared of these cords three hours after asphyxiation showed degenerative changes in the motor cells, which eventually resulted in their total destruction (van Harreveld and Tyler, 1942). It was therefore of special interest to examine the changes in cord polarisation after such long asphyxiations.

In typical experiments of this kind it was found that when the aortic clamp was released, there was at first an increase in the negativity of the gray matter. After this the galvanometer moved very slowly towards the pre-asphyxial position for 1 to 1½ hour, without reaching it, however. The potential remained stationary for the next one or two hours after which the negativity of the gray matter increased again to reach a new level in about an hour. This course of the potential would indicate a temporary repolarisation of elements in the gray matter, which is well in accordance with the observation of temporary reflex action followed by destruction of nerve cells in the gray matter.

The changes in potential after long asphyxiations stretch over many hours, requiring for their recording extraordinary constancy of the preparation as well as of the apparatus. Therefore a less exacting way was sought to examine the state of polarisation of the gray matter after such asphyxiations. It is obvious that short asphyxiations under these circumstances will yield potential changes only when, and so long as the gray matter elements are polarised. Thus by clamping the aorta for 1 minute periods at intervals of 20 to 30 minutes, it is possible to investigate the state of polarisation of the gray matter after long asphyxiations. Figure 5 gives the results of such an experiment. One minute clamping of the aorta, 30 minutes after a 70 minute asphyxiation of the cord produced the record A; records B, C, D, E, F, and G were made the same way at half-hour intervals. The depolarisation potential produced after 30 minutes (A) is quite small, half an hour later (B), however, it has grown considerably. For the next hour the state of polarisation of the gray matter remains the same as indicated by the depolarisation potential (C and D). Two and a half hours after the end of asphyxiation 1 minute clamping still causes a large effect, which, however, shows a delayed recovery (E). Half an hour later clamping has very little effect (F), and if this effect is real, no recovery occurs. The record G, made 3.5 hours after the end of asphyxiation shows no depolarisation potential. The tardy recovery as recorded in E, shortly before the disappearance of the depolarisation potential, has been observed in other experiments. There is no reason to assume that this delayed recovery is caused by circulatory insufficiency

and it is believed that it is the expression of the impending destruction of gray matter elements.

Not after all 60 and 70 minute asphyxiations did the effect of 1 minute clamping of the aorta disappear completely during the time available for observation (3 to 4 hrs.). It is possible that in these experiments the period of observation was insufficient for the cell destruction to have proceeded far enough. It is also possible, however, that other depolarisable structures like the dendrites are responsible for these small remains of the depolarisation potential. As mentioned above, even 2 to 3 days after 60 to 70 minute asphyxiations small depolarisation potentials have been observed.

Survival time of reflex activity. It seemed of interest to compare the course of cord depolarisation during asphyxiation with the survival time of reflex activity. This value was therefore determined for asphyxiation of the cord by respiratory arrest, using the kneejerk as indicator. In 26 asphyxiations on 12 animals the

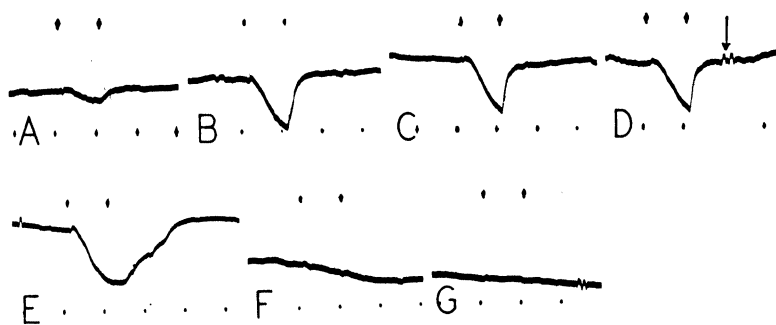


Fig. 5. Series of 1 minute clampings of the aorta at 30 minute intervals, made after a 70 minute asphyxiation of the cord. The two notches at the arrow in D show the deflection for 1 millivolt. Upper signal shows beginning and end of clamping of the aorta, lower signal indicates time in minutes.

kneejerk disappeared after an average of 164 seconds. The longest and shortest survival times were 101 and 241 seconds.

DISCUSSION. Extraspinal causes of the potentials recorded during asphyxiation are excluded by the absence of these effects two weeks after a 60 to 70 minute cord asphyxiation.

Anoxia (by breathing nitrogen) and asphyxia (by respiratory arrest or by clamping the aorta) produce similar potential changes, though the carbon dioxide accumulation in the cord can be expected to be quite different. It is therefore unlikely that pH changes in the cord cause the potentials recorded during asphyxiation. Also the slow potential changes after long asphyxiations indicate a slow recovery of damaged elements, rather than the removal of a metabolite.

The absence of electrical effects 10 to 14 days after 60 to 70 minute cord asphyxiation points to the neural elements as the structures producing the potentials recorded during asphyxiation, since such long asphyxiations destroy nerve cells (van Harreveld and Tyler, 1942) and by subsequent Wallerian de-

generation the fibres attached to them. The electrical effects of cord asphyxiation support in general the postulate presented in the introduction, that they would be caused by an inequality in sensitivity of various neuron parts to oxygen lack; the nerve cells (and perhaps the dendrites and bouton terminaux), which have the higher metabolism (Holmes, 1930), being depolarised the earliest. The decrease or even disappearance of depolarisation potentials some hours after a 60 to 70 minute clamping of the aorta (fig. 5) which coincides with the appearance of degenerative changes in the motor cells (van Harreveld and Tyler, 1942) points to the depolarisation of the nerve cells as the most important factor in the production of the depolarisation potential.

The mechanism of the potential lead off from the cord is relatively simple only during the first few minutes of asphyxiation, when the nerve cells (and perhaps the dendrites) are the only depolarising structures. After these first few minutes, however, the white matter also begins to depolarise, which may cause the decrease in the potential, present in all but a few of the records obtained during prolonged clamping of the aorta². The initial increase in negativity of the gray matter observed during the recovery from long asphyxiations supports this assumption. It is likely that the white matter which depolarises slower than the nerve cells, recovers and repolarises again quicker after reoxygenation of the cord, thus restoring a potential difference between gray and white matter, which will decrease again with the repolarisation of the nerve cells. According to this view the initial increase of the gray matter negativity can be expected only after the longer asphyxiations, since only these produce white matter depolarisation. In accordance with this, initial increase of gray matter negativity has never been observed after 5 minute asphyxiations and only once in three 10 minute clampings of the aorta.

The depolarisation potential after the first few minutes of asphyxiation has to be regarded as a complex phenomenon, and therefore cannot be considered as a reliable indicator of the state of polarisation of the gray matter elements. Thus, the potential maximum reached after a few minutes does not necessarily indicate the complete depolarisation of the nerve cells; it may, e.g., indicate the beginning of white matter depolarisation. This nature of the depolarisation potential may further explain why the maximum potentials recorded during the first minutes of asphyxiation sometimes differ with different methods of cord asphyxiation. In one cat, e.g., clamping the aorta produced a maximum potential of 5.1 millivolts, whereas asphyxiation by respiratory arrest caused a maximum of 12.4 millivolts. In another animal these values were 4.9 and 12.5 millivolts. A difference in the relative speed with which the cells and the axons depolarise with the two methods of asphyxiation offers an explanation for these observations.

On the average the kneejerk was abolished in less than three minutes after respiratory arrest, while the potential maximum was reached after about 4

² The cord remained negative with respect to the root in all experiments. This is probably due to the use of the special electrode in which the anterior root was kept oxygenated and thus polarized.

minutes under these circumstances. Even if this maximum is probably not the expression of the complete depolarisation of the nerve cells, a comparison of these values indicates that depolarisation has progressed considerably before reflex conduction is abolished.

Two periods of tone have been observed after 65 minute asphyxiations (van Harreveld, 1943, 1944 b); an initial tone which is quite weak and which develops and disappears usually between 10 and 35 minutes after the end of asphyxiation, and a much stronger secondary tone, which may develop at varying times after the initial tone, usually after 1 to 2 hours. Eventually this tone also disappears and from then on the hind legs remain flaccid. Tone can be produced in the toneless period between initial and secondary tone by renewed cord asphyxiation;

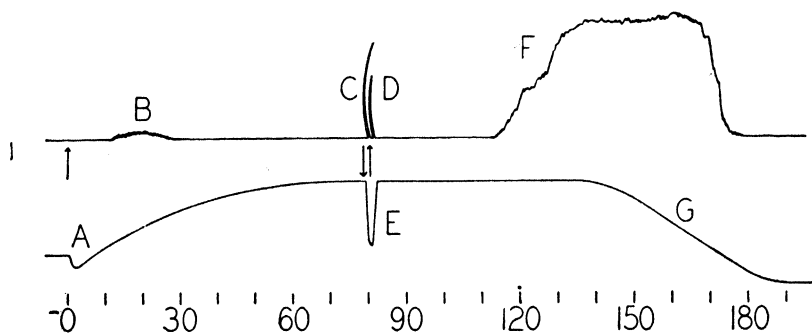


Fig. 6. Scheme of the relation between tone and potential changes observed after a long (60 to 70 min.) asphyxiation. Left arrow indicates end of asphyxiation. A, increased gray matter negativity, indicative of white matter repolarisation. After this the gray matter becomes slowly less negative indicating repolarisation of gray matter elements. B is initial tone. C and D indicate the periods of tone observed during the development and recovery from a short asphyxiation (indicated by arrows) during the toneless period between initial and secondary tone, which causes the depolarisation E. F is the secondary tone. G depolarisation caused by the destruction of nerve cells. Time in minutes.

a short period of tone is observed regularly during its development, and in some instances also during the recovery from asphyxiations of suitable duration (van Harreveld, 1943). Synaptic conduction has been demonstrated during most of these periods of tone.

In figure 6 it has been attempted to correlate the periods of tone observed after 65 minutes of asphyxiation with the state of polarisation of the gray matter elements, as indicated by the potential changes observed after these long asphyxiations. It is certain that the initial tone occurs during repolarisation which is indicated by the decreasing negativity of the gray matter. It has been shown that short asphyxiations during the time corresponding with the toneless period causes a depolarisation with prompt repolarisation on re-oxygenation of the cord (fig. 5). The two short periods of tone which can be observed during such a short asphyxiation probably occur during the depolarisation and repolarisation of the gray matter elements. After long asphyxiations it thus

seems that neither in the depolarised gray matter, nor in the cord polarised as fully as circumstances allow, reflex conduction is possible. Synaptic conduction has been observed only during the transition from the depolarised into the polarised state and vice versa.

The secondary tone coincided roughly with the depolarisation caused by the destruction of the nerve cells. It is questionable, however, whether the depolarisation alone can explain this usually strong tone. The recovery of reflex conduction in a disinhibited reflex arc, postulated before (van Harreveld and Marmont, 1942), is probably also a factor in the production of the strong secondary tone.

Many authors described enhanced reflex activity during cord asphyxiation (for references see van Harreveld, 1944 a). Gesell and Hansen (1945) recently explained this on the basis of an anticholinesterase activity of the increasing acidity of the gray matter during asphyxiation. The facilitating influence on reflex conduction of partial depolarisation is offered here as an alternate explanation for this phenomenon.

I am indebted to Mrs. J. Wiersma, Miss R. E. Estey and Dr. E. B. Wright for valuable assistance.

SUMMARY

The potential differences which develop between the gray matter and the anterior root during asphyxiation of the spinal cord were recorded. These potential changes are believed to be the expression of the depolarisation first of the nerve cells, and later of the nerve fibers in the cord.

After the arrest of respiration the gray matter starts to become negative after about 50 seconds. After feeding nitrogen into the apparatus for artificial respiration the negativity began after about 20 seconds and after clamping the aorta after 8 to 9 seconds. These differences are explained by the different speeds with which the spinal cord is deprived of oxygen. The negativity reaches a maximum in 2 to 4 minutes after which it usually slowly starts to regress. The negativity of the gray matter is probably due to the depolarisation of the cells, the regression to the subsequent depolarisation of the fibers in the cord.

The negativity of the gray matter is quickly reversible when the cord is supplied with oxygen again. The recovery starts after about 20 seconds following a 1 to 2 minute arrest of respiration or breathing of nitrogen. After 1 to 5 minute clamping of the aorta the recovery starts even quicker, namely, after an average of 9 seconds.

After longer asphyxiations of the cord by clamping the aorta (10 to 70 min.) the negativity of the gray matter also regresses after reoxygenation of the cord, though often not completely. In such experiments this regression is often preceded by a temporary increase of the negativity of the gray matter, which is believed to be due to the repolarisation of the nerve fibers before the cells recover.

After the longest asphyxiation (60 to 70 min.) it was found that the cells in the spinal cord first repolarise, but a few hours later depolarise again. This is in

accordance with previous observations which showed that the cells in the cord are destroyed a few hours after the end of such long asphyxiations.

The relation between the state of polarisation of the cells and reflex activity is discussed.

REFERENCES

- BRONK, D. W., M. G. LARRABEE AND P. W. DAVIES. *Fed. Proc.* **5**: 11, 1946.
 COWAN, S. L. *Proc. Roy. Soc. London* **115B**: 216, 1934.
 FURUSAWA, K. *J. Physiol.* **67**: 325, 1929.
 GERARD, R. W. *This Journal* **92**: 498, 1930.
 GESELL, R. AND E. T. HANSEN. *This Journal* **144**: 126, 1945.
 HOLMES, E. G. *Biochem. J.* **24**: 914, 1930.
 KOCH, E. *Pflüger's Arch.* **216**: 100, 1927.
 ROSEMAN, E., C. W. GOODWIN AND W. S. MCCULLOCH. *J. Neurophysiol.* **9**: 33, 1946.
 SPIEGEL, E. A., G. C. HENNY, H. T. WYCIS AND M. SPIEGEL-ADOLF. *This Journal* **146**: 12, 1946.
 VAN HARREVELD, A. *This Journal* **139**: 617, 1943.
 This Journal **141**: 97, 1944a.
 This Journal **142**: 428, 1944b.
 VAN HARREVELD, A. AND G. MARMONT. *J. Neurophysiol.* **2**: 101, 1939.
 VAN HARREVELD, A. AND D. B. TYLER. *This Journal* **138**: 140, 1942.

APPENDIX

A DIFFERENTIAL DIRECT CURRENT AMPLIFIER

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Figure 1 shows a convenient, simple, differential direct current amplifier which can be used with an inexpensive microammeter in the output circuit to combine good sensitivity, stability and protection from overloads. The input tubes¹ are a type developed for electrometer service and are tested for stability, uniformity of characteristics under the operation conditions here employed, and for grid current. The latter is held below 10^{-12} amperes. Consequently, electrode polarization from grid current is negligible in the absence of large AC pickup.

Response to direct current or low frequency AC voltages common to both input leads is minimized by feedback as follows. If the grids of the input tubes both become more positive their plate potentials become more negative, decreasing the plate currents of the second stage triodes and the voltage drop across the resistor between their cathodes and the filament of the first stages. The cathodes are at fixed voltage relative to ground, consequently the filaments of the first stages must follow the potential change of the input grids, causing a decrease in the input potentials on this stage.

¹ Part no. 932, National Technical Laboratories, South Pasadena, Calif. Available from most laboratory supply houses.

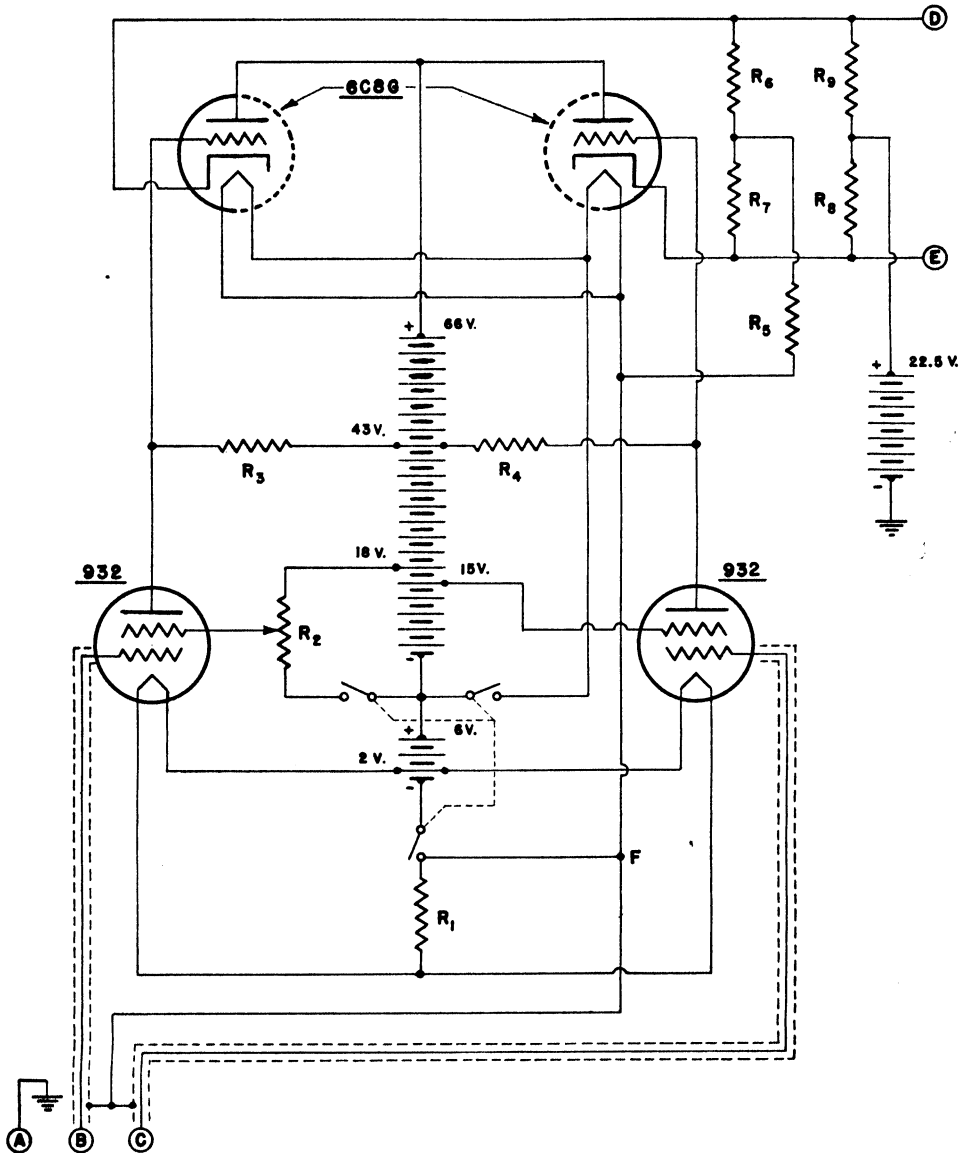


Fig. 1. Differential amplifier circuit. R_1 , 14 ohm wirewound resistor. R_2 , ten-turn Helipot, 5,000 or more ohms (The Helipot Corporation, South Pasadena, Calif.). R_3 , R_4 , 100 megohms (S. S. White Dental Mfg. Co., Industrial Division, New York 16, N. Y.). R_5 , 25,000 ohms. R_6 , R_7 , R_8 , R_9 , 5,000 ohms. Six volt storage battery for filament supply. Remaining batteries, medium size "B" batteries.

In addition, the amplifier has the usual advantages of "balanced" circuits (1). The practical improvement will vary with the degree to which all elements including the tubes in the circuit are symmetrical, but thanks to the feedback

and to the low current drain on the batteries a useful degree of stability is achieved without elaborate tube matching or circuit balancing.

The differential transconductance of the entire amplifier is about 0.05 mhos, that is, a one millivolt signal will produce a change of output current through a low resistance load across terminals *D* and *E* of about 50 microamperes. This follows from the facts that the amplification of the first stages is about 50 times, and the transconductance of the output stages is about 1000 micro-mhos.

The maximum output current available is limited to about one milliampere. It may be further decreased by lowering the plate voltage to the output stages and simultaneously increasing *R*₅, but at some risk of encountering objectionable non-linearity within the normal operating range. High sensitivity indicating devices can be used, but should be protected.

A conventional standardizing circuit may be used in series with either input terminal (*B* or *C*). It should be well insulated and shielded, and should have low capacity to ground to avoid unbalance of input circuits for power line frequency.

In construction of the amplifier, good insulation and protection from high humidity are essential for stability. Batteries in good condition will minimize drift and "noise." If on first trial the output cannot be brought to zero by varying *R*₂ with the input terminals shorted, interchange the no. 932 tubes.

It is advisable after assembling the complete equipment to check the AC voltage appearing between circuit point *F* and ground with an oscilloscope or a high impedance AC voltmeter. Input terminals of the amplifier should be connected to a typical preparation. If more than about one-tenth volt RMS is found, the shielding should be improved.

REFERENCE

- (1) BURR, H. J., C. T. LANE AND L. F. NIMS. Yale J. Biol. and Med. 9: 65, 1936.

LIMB BLOOD FLOW AND VASCULAR RESISTANCE CHANGES IN DOGS DURING HEMORRHAGIC HYPOTENSION AND SHOCK¹

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This report concerns itself with changes in blood flow through the unoperated limbs of dogs during the course of hemorrhagic hypotension and circulatory failure subsequent to reinfusion of blood. The technique for production of such states has been previously described (1). Briefly, it consists in bleeding a dog until mean arterial pressure is kept at 50 mm. Hg for 90 minutes, and at 30 mm. Hg for another 30 minutes. Then all the heparinized blood withdrawn is reinfused and circulatory failure awaited.

Two purposes are served by these studies: 1, they supply evidence as to whether compensatory changes in anesthetized dogs differ quantitatively or qualitatively from those which occur clinically, and 2, they yield basic information regarding reactions of the circulation in skin and muscles after transfusion of blood when irreversible shock has developed. It is important that flow studies be made on a limb which has not been submitted to operative procedures and which is protected from possible reflexes from an opposite extremity or other sources. Most of the previous work instituted to evaluate resistance changes in limbs of experimental animals has involved surgical manipulation of femoral vessels or tissues in their vicinity; some has in addition involved perfusion of leg vessels with the pitfalls incident to these methods.

In these experiments such complications were avoided and natural conditions were maintained by adapting the plethysmographic method and technique described by Wright and Phelps (2) in the study of human blood flow. In this way not only rate of flow but the characteristics of the pulse flow—systolic forward flow, systolic backflow, and diastolic forward flow—could be determined. In addition, the changes in volume of the other hind leg were followed in some experiments by use of a water plethysmograph. In most experiments flow changes were correlated with temperature changes in various structures of the leg.

METHODS. This study was made on 22 dogs under morphine and barbital anesthesia. The neck was opened and an intratracheal cannula was inserted. Mean and optical blood pressures were recorded from the left common carotid artery. To maintain intact femoral vessels bleeding was done through the right common carotid or right axillary artery and the blood was reinfused into the right external jugular vein. In most cases a warm animal board maintained rectal temperature within 1°C. throughout the experiment. The right hind leg was

¹ These studies were supported by a grant from the Commonwealth Fund.

shaved and its volume determined by the water displacement method. The air-filled plethysmograph as described by Wright and Phelps (2) was adapted for use on the dog (fig. 1). A ligature, *E*, through the right paw enabled the leg to be drawn and fastened within the chamber. An airtight nonconstrictive seal was afforded by the use of melted Unna paste. The position of the plethysmograph was meticulously adjusted so as to avoid movements of the leg during inspiration. Flow measurements were made only between respirations. An effort was made to maintain a constant temperature within the chamber.

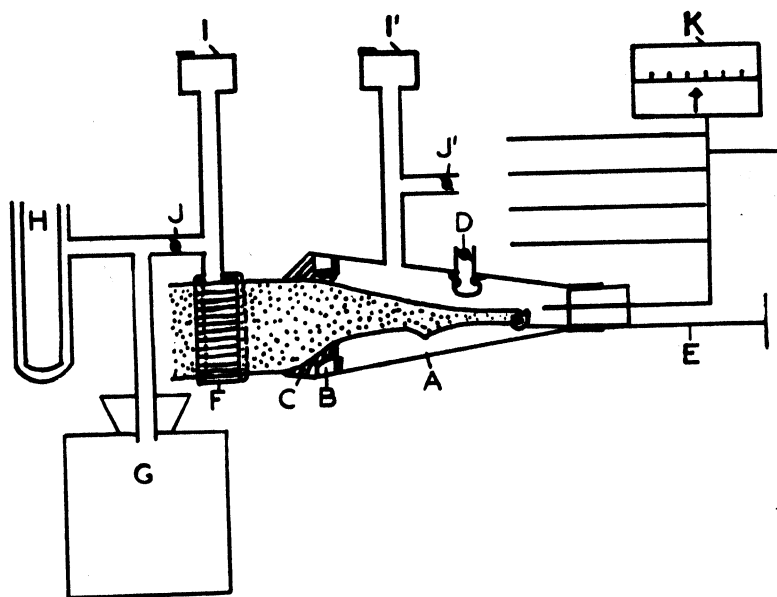


Fig. 1. Shows plethysmograph and cuff in place and Micromax temperature recorder. *A*, plethysmograph; *B*, plaster of Paris; *C*, Unna paste; *D*, calibrating device consisting of metal tube with small rubber bag and stopcock (fluid was injected from calibrated syringe); *E*, ligature holding leg in place; *F*, cuff; *G*, pressure bottle; *H*, Hg manometer; *I*, optical manometer for recording time of cuff inflation; *I'*, optical segment capsule measuring leg volume change; *J*, stopcock controlling inflation of cuff; *J'*, stopcock opening and closing plethysmograph to outside; *K*, Micromax temperature recorder.

In an effort to evaluate and control the vasomotor reactions induced by anesthesia and external temperature changes (3) and to correlate blood flow with cutaneous and muscle temperature, a Leeds and Northrup six point micromax recorder was used simultaneously to record temperatures from paw, muscle, skin, rectum, room and plethysmograph.

Rate of flow was measured by opening stopcocks *J* and *J'* (fig. 1) electrically with an x-ray timer, thereby simultaneously inflating cuff *F* to a pressure slightly below the diastolic existing at any time during an experiment and opening the plethysmograph to the outside. One second later stopcock *J'* was automatically closed by the electrical arrangement. The leg volume change due to the inflow of

blood was measured by a segment capsule 25 mm. in diameter, I' . A test reaction reproduced in figure 2-A shows that even when the femoral artery is compressed inflation of the cuff displaces 1.6 cc. into the enclosed leg in 0.66 second, thereby proving the necessity of an open plethysmograph during cuff inflation (2).

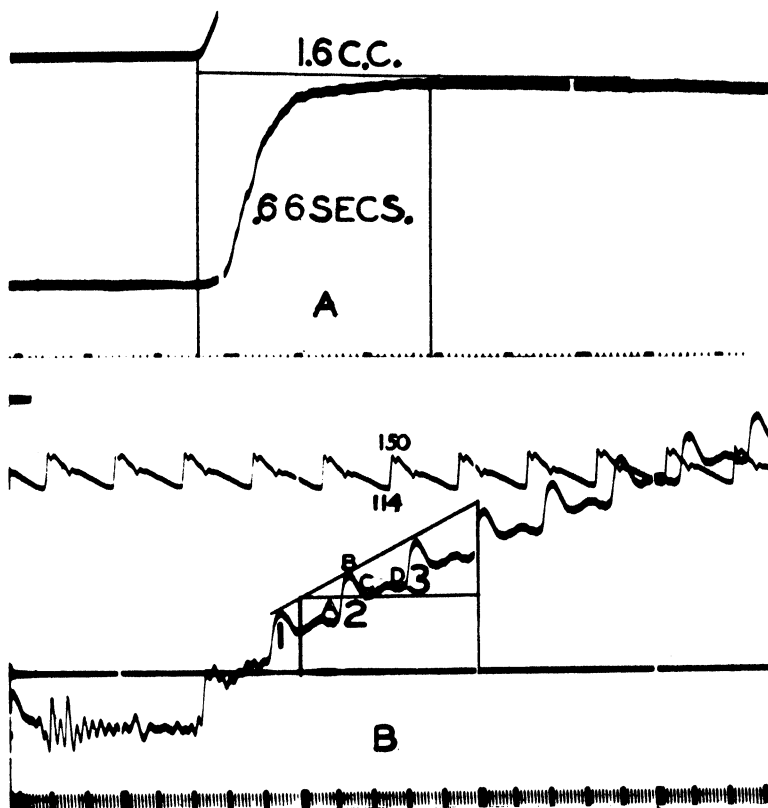


Fig. 2. A. Shows effect of cuff inflation upon volume of enclosed hind leg when plethysmograph is closed during occlusion of femoral artery. Upper line—time of cuff inflation; middle line—volume of enclosed leg; lower line—time.

B. Typical flow record showing method of line construction for flow measurement. Upper left—cuff inflation marker; upper—carotid pressure pulse; middle—leg flow, showing 1st, 2nd and 3rd beats described in text (*a-b*, systolic inflow, *b-c*, systolic back flow, *c-d*, diastolic flow); lower—time in seconds, $\frac{1}{2}$ second and $\frac{1}{10}$ second.

Figure 2-B is a typical flow record of a dog's hind leg. It shows that the rate of rise begins to diminish after the third pulse flow curve and often after the second. The direction of the drawn line gives the probable rate of flow, the deviation of the pulse summits from this line represents an artefact of flow inherent in the procedure. This artefact may be due in some degree to the slightly increasing pressure within the system when a Frank capsule is used, but since it also occurs

with pure volumetric registrations (4) it is probably due chiefly to filling of venous channels and the resistance which this affords to capillary runoff. We therefore agree with previous studies (2, 4) that the rate of blood flow cannot be determined by the rising line of crude recorders writing with ponderable levers because they are unable to detect this artificially diminishing flow.

It is obviously important, as Wright and Phelps (2) suggest, that flow evaluations be computed from the first or second curve. In order to expedite measurements, our technique varied from that suggested by them (2) in that flow measurements were made by applying the calibration scale to the rise of a line over a one second period which was drawn to connect equivalent points of the first two normal pulses. When multiplied by 60 and divided by the volume of the inclosed leg the result is rate of flow per minute per 100 grams of leg. This flow record shows the flows during single heart beats, and from an early pulse systolic flows (*a-b*) and back flows (*b-c*), as well as diastolic flows (*c-d*), may be calculated. This record further shows a diminution in net forward flow with each successive beat. This is first in evidence in the third beat and calculations show that the first and second beats have an equal diastolic forward flow. In later beats, however, an increased systolic back flow and a decreased diastolic forward flow become apparent.

In some experiments volume changes in the other hind leg were estimated. The air filled plethysmograph was found to be unsatisfactory because of large volume changes induced by small temperature changes, and therefore a saline filled plethysmograph was used. Since Unna paste is water soluble, effective sealing was accomplished with commercial "tree tanglefoot," water pump grease, or caulking compound. The chamber was constructed from a glass cylinder. After the hair about the knee had been removed a ligature through the paw enabled the leg to be drawn into and anchored within the chamber. The remaining hair was carefully wet with soap and water to minimize the formation of bubbles. A long, small bore, horizontal, calibrated glass tube was inserted in the distal end of the cylinder by means of a rubber stopper. The chamber was filled with saline and air allowed to escape through a second glass tube in the rubber stopper. The chamber was anchored in place and further bubbles removed through a long needle inserted through the sealing compound. Volume changes were determined by recording changes of the fluid column in the calibrated tube.

Two to 2½ hours were allowed to elapse after anesthetization before control leg flows, blood pressure, heart rates, temperatures, and leg volumes were taken. After bleeding, similar data were repeatedly obtained in both the 50 and 30 mm. Hg periods. During the 30 mm. Hg period artificial respiration was often needed. After reinfusion further data were collected about every ½ hour until death.

Since we hoped to learn something of resistance changes to blood flow and since Green (5) pointed out that deductions made by simple pressure/flow ratios are questionable when blood pressure is not constant, an effort was made in some experiments to maintain a constant blood pressure in the post-infusional period. This was done by withholding a part of the blood and infusing only as necessary

to maintain the desired blood pressure. Finally, in order to adjust the survival time to the needs of these experiments the hypotensive periods were at times prolonged and at other times shortened, as appeared advisable.

RESULTS. Of the 22 dogs used for these experiments 5 were controls, one died during the hypotensive state, one died during the reinfusion of blood, 4 lived longer than 6 hours, and 11 developed typical shock. The survival time of these 11 dogs after reinfusion was as follows: 1 lived 4 hours, 3 lived 3 hours, 1 lived 1½ hours, 2 lived 2 hours, 1 lived 1 hour, and 3 lived 30 minutes. There was in general an inverse relationship between the rectal temperature and time of survival.

Figure 3 shows the trend of events in a typical experiment. In this animal the 50 mm. period was maintained for 30 minutes and the 30 mm. period for 90 minutes. The control data (C) show the heart rate to be 175 and the mean blood pressure 145. The control flow was 8.1 cc. per minute per 100 grams of leg. P/F is the ratio between mean blood pressure and flow per minute per 100 grams of leg. The control value is 13. The control rectal temperature is 36.6, and it remains between 36 and 37° throughout the experiment. The control paw temperature was 36.5, muscle temperature was 36.0, and room temperature varied between 22 and 22.8°.

During period B, bleeding was carried out and mean blood pressure fell gradually to 50 mm. The heart rate increased slightly at first, but later declined. Blood flow fell quickly and the P/F ratio rose rapidly. Paw and muscle temperatures were maintained until the end of the bleeding period. During the 50 mm. period (labeled "50") the heart rate further decreased and then increased. At the end of the period there was an additional slight decline in flow and a further rise in the P/F ratio. Paw and muscle temperatures both fell, that of the paw far more than that of muscle.

During the 30 mm. period (labeled "30") the heart rate was constant except toward the end of the period. The flow fell still further with a slight temporary increase in the latter half. The P/F ratio continued its marked rise with a temporary fall during the last half of the period. The paw temperature fell still more, as did muscle temperature.

Upon reinfusion at *I*, there was a fall in heart rate, a rise in blood pressure, a marked rise in flow, a marked fall in P/F ratio, with a rapid increase in paw and muscle temperature. Following reinfusion the heart rate increased slowly, the blood pressure rose gradually to 150 mm. Hg in 1½ hours. The blood flow rapidly rose to above the control value before the blood pressure reached its maximum. However, while the blood pressure was still increasing the flow decreased markedly. The P/F ratio decreased slightly after reinfusion and then increased to above the control. Paw and muscle temperatures continued to increase.

Terminally, labelled *S*, there was a gradual fall followed by a rapid decline of blood pressure, a further decrease in flow and an increase in the P/F ratio to the very end, and a fall in paw temperature.

A more careful analysis of the data from this experiment reveals three impor-

tant facts. First: Following reinfusion, even while the mean blood pressure is still rising, the flow decreases and the P/F ratio increases. Second: Except for the early and brief post-infusional increase in flow and decrease in P/F ratio there is a consistent and prolonged decrease in flow and increase in P/F ratio to

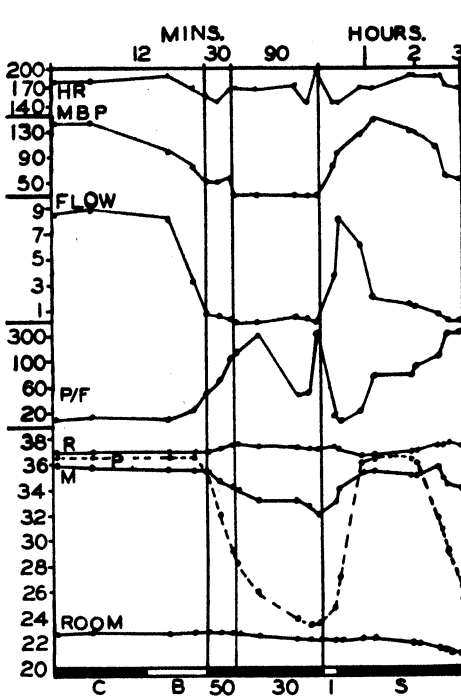


Fig. 3

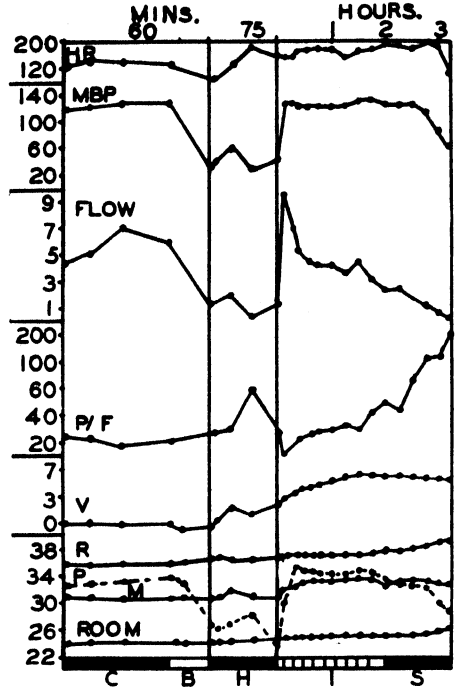


Fig. 4

Fig. 3. Shows data from typical experiment. Top is time. Left shows values for the following: *H.R.*, heart rate; *M.B.P.*, mean blood pressure in millimeters Hg; flow in cubic centimeters per minute per 100 grams of leg; *P/F*, mean blood pressure/flow per minute per 100 grams of leg; *R*, rectal temperature in Centigrade; *P*, paw temperature; *M*, muscle temperature; room temperature. Bottom—*C*, control, *B*, bleeding period, 50 mm. period, 30 mm. period; *I*, infusional period; *S*, post-infusional and period of shock.

Fig. 4. Data from second experiment with leg volume values. Top is time. Left margin shows values for following: *H.R.*, heart rate; *M.B.P.*, mean blood pressure in millimeters Hg; flow in cubic centimeters per minute per 100 grams of leg; *P/F*, mean blood pressure/flow per minute per 100 grams of leg; *V*, leg volume change in cubic centimeters; *R*, rectal temperature in degrees Centigrade; *P*, paw temperature; *M*, muscle temperature; room temperature. Bottom: *C*, control; *B*, bleeding period; *H*, hypotension period; *I*, period of multiple small infusions of blood; *S*, post-infusional and period of shock.

the very end. Third: A comparison of flows and P/F ratios in the post-infusional and control periods reveals that the post-infusional flows are less and the P/F ratios greater than in the control period despite equal mean blood pressure levels.

The dog from which these data were collected showed a marked hemorrhagic diarrhea shortly after reinfusion and at autopsy the duodenum showed severe mucosal hemorrhage.

A composite graph of several experiments not illustrated shows variations, but in general the same pattern of events occurs. In one experiment the flows and P/F ratios are variable and the animal survived the longest. In some experiments there was a terminal, temporary increase in blood flow which was in the control range in spite of a lower mean blood pressure. This was associated with a lower P/F ratio.

Figure 4 shows the data from our most successful effort to maintain a constant post-infusional mean blood pressure. This pressure was maintained between 120 and 127 mm. Hg for $2\frac{1}{2}$ hours, which level was likewise the control range. Early in the post-infusional period the flows and P/F ratios were within the control range, but in the latter half, despite constant mean blood pressure, the flows decreased and P/F ratios increased. The leg volume changes in this experiment are also plotted. There was a slight fall during the early bleeding period, but then a slow, progressive increase to 6.1 cc. per 100 grams of leg which fell only slightly during the last half hour of life. In two other dogs in which leg volumes were successfully measured the trend of events was similar. Both dogs died within an hour and the increases in leg volume were 2.0 and 2.5 cc. respectively. A fourth dog which was still alive after $3\frac{1}{2}$ hours showed a leg volume increase of 4.5 cc.

Backflows. In some experiments systolic and diastolic backflows were calculated and compared with forward and net flows during the various stages of the experiments. In all cases a systolic backflow existed during the control which approached or became zero during the bleeding and hypotensive periods. After reinfusion there was always an increase in systolic backflow to levels sometimes above the control. The maximum increase, however, was not immediate, but was reached after the net forward flow had decreased and the P/F ratio increased. The systolic backflow then progressively decreased until death.

Diastolic control flows were forward but became backflows during bleeding. Upon reinfusion these flows became positive and remained so, some increasing and others decreasing.

In addition to the fall in blood pressure and death other constant criteria of hemorrhagic shock were the hemorrhagic diarrhea and the intestinal mucosal hemorrhage. These findings have come to be accepted as important criteria for this type of shock.

DISCUSSION. Pallor of the skin and cold extremities are considered outstanding clinical signs of shock. The inference that this denotes diminished circulation has been confirmed by blood flow measurements in man (6). Severe reduction in blood flow through the limbs has also been demonstrated in anesthetized animals in various forms of experimental shock, and has been attributed to throttling of blood flow by increased peripheral resistance (7, 8, 9). A demonstration that similar reactions occur in animals submitted to standardized bleeding technique outlined above would indicate that the compensatory vascular reactions are similar to and the conditions comparable to those which develop in clinical forms of shock.

Studies on relatively intact animals submitted to standard periods of hemor-

rhagic hypotension and transfusions of their own blood have shown that in some dogs the circulatory failure which eventually develops is accompanied by decreasing total peripheral resistance (10). About this time also vasomotion ceases in observed vessels and venous congestion begins to develop in the meso-appendix (11). However, decrease in total peripheral resistance is by no means a universal occurrence. "Despite the universally fatal outcome, changes in T.P.R. were extremely variable during periods of posthemorrhagic hypotension and during circulatory failure which developed after reinfusion of blood" (10). It is conceivable, however, that regional resistances, e.g., in skin and muscles, may be reduced considerably without necessarily affecting or decreasing T.P.R. If, as has been postulated, constriction of surface vessels serves the compensatory purpose of circulating a greater proportion of the available blood volume through internal organs, reduction of resistance in these territories—either through vasodilatation or reduction of muscle tonus and tissue pressure—might abrogate an important compensatory mechanism and thus lead to circulatory failure.

We have already pointed out that leg flow decreases and the P/F ratio increases in the post-infusional period even when mean blood pressure is still increasing or while it is maintained. Likewise, it is usual for the post-infusional flows to be less and the P/F ratios greater than found in the control period with equal mean blood pressures. To us these facts must be interpreted to mean increasing resistance to flow during the post-infusional period. We cannot explain why the changes occur in the face of increasing blood pressure, and we are unable to call upon obvious need for compensatory mechanisms which at any rate are still viable. Known changes in hematocrit values following hemorrhage do not seem to increase blood viscosity sufficiently to offer such increasing resistance to flow. Since these reduced flows are found when mean blood pressures are constant or equal to control values we do not believe that the objections of Green (5) apply to these measurements.

However, other variables discussed by Green (3) are more difficult to control, namely, those of the temperature control mechanism. He deduces from his findings that one should wait 3–4 hours after morphine and barbital anesthesia before making flow determinations. Our controls were made 2 to 2½ hours after anesthetization during which time the animal was heated and the paw and rectal temperatures were constant or slowly rising. At the time of reinfusion the rectal temperature was practically always still higher and during the development of shock it was approaching or had reached the 39–40° range at which Green found the constriction of the paw vessels to disappear. Also, this post-infusional period of observation was always beyond the four hour period which he established for adjustment of temperature. Finally, this is a study of shock which clinically is known to reduce body temperature. It is not too far afield to suggest that in man part of the picture of shock, with its signs of cutaneous constriction, may be a compensatory effort to maintain body temperature.

The question may be raised as to the relative amounts of skin, muscle, and bone concerned in these flow measurements. A dissection was made of that portion of a leg enclosed within the plethysmograph and skin, muscle and bone

were weighed. It was found that skin comprised 18 per cent, muscle 32 per cent, and bone 50 per cent. Since by this method blood flow through bone was probably not measured we may assume that $\frac{2}{3}$ of our measured blood flow is through muscle, fascia and tendon, while $\frac{1}{3}$ is through skin.

It was realized early that skin and muscle temperatures changed together in the same direction as paw temperatures, but these changes were of smaller magnitude. We concur in the interpretation of Green (3) that these temperatures are influenced by the temperature of underlying structures and hence are more apt to follow rectal temperature. The paw, however, is isolated from general body temperature and therefore gives a far better indication of changes in blood flow. It has been observed (records not shown) following sciatic nerve section and subsequent stimulation of its peripheral end that paw temperature rapidly indicates increases in blood flow, but responds more slowly to decreases in flow. In figure 3 this is also evident, for even after the flow in the post-infusional period has declined the paw temperature shows a slight increase and does not show a marked fall until later. It is evident, therefore, that although paw temperature gives better indication of flow than skin or muscle temperature, nevertheless rapid changes in blood flow cannot be estimated from paw temperature changes.

Successful measurements of leg volume changes in 3 dogs dying in shock indicate increases of from 2 to 6.1 cc. per 100 grams. A fourth dog, still alive after $3\frac{1}{2}$ hours with no signs of shock, showed a leg volume increase of 4.5 cc. per 100 grams. It would seem therefore that the loss of fluid to tissue or the amount of fluid trapped within vessels in limbs bears no relation to the development of shock. Calculations, although hazardous, would indicate that a total loss of circulating fluid of 400 to 500 cc. may occur provided such measured loss in the leg is generalized.

The conclusion is reached, then, that there is no evidence from these experiments that in hemorrhagic shock there is dilatation of the arterial network of the legs since there is diminished blood flow, fall in paw temperatures and rising resistance to flow. The increase in leg volume does not necessarily indicate that loss of circulating fluid is part of hemorrhagic shock.

SUMMARY

1. Changes in rate of blood flow through limbs of anesthetized dogs were determined by an adaption of the Wright-Phelps technique during the course of prolonged sustained post-hemorrhagic hypotension, reinfusion, and post-reinfusional circulatory failure. Alterations in the ratio of pressure/flow were determined and their significance as criteria of changes in peripheral resistance were discussed.

2. Immediately after bleeding the blood flow decreases abruptly and the pressure/flow relationship is altered very little. Subsequently, blood flow decreases somewhat more and the pressure/flow ratios increase greatly. While periodic reversals occur the main trend appears to be in the direction of increased resistance to flow.

3. Following reinfusion, blood flow exceeds control rates for a short while,

but soon it decreases progressively while arterial pressure remains high or even rises. The tendency toward increased resistance continues during subsequent decline of blood pressure.

4. Supplementary studies of volume changes of a limb during the entire course of our experiments reveal volume increases ranging from 2 to 6.1 cc. per 100 grams.

5. The results give no evidence *a*, that circulatory changes in the limbs of dogs differ from those observed in clinical shock; *b*, that reduction in resistance in the limbs or loss of circulating fluid into limbs plays a significant part in the circulatory failure which follows reinfusion of blood into animals exposed to prolonged periods of hypotension.

REFERENCES

- (1) HUIZENGA, K. A., B. L. BROFMAN AND C. J. WIGGERS. *J. Pharmacol. and Exper. Therap.* **78**: 139, 1943.
- (2) WRIGHT, G. W. AND K. PHELPS. *J. Clin. Investigation* **19**: 273, 1940.
- (3) GREEN, H. D., N. D. NICKERSON, R. N. LEWIS AND B. L. BROFMAN. *This Journal* **140**: 177, 1943.
- (4) ROTHLIN, VON E. AND A. CERLETTI. *Helv. Physiol. Acta* **4**: 135, 1946.
- (5) GREEN, H. D., R. N. LEWIS, N. D. NICKERSON AND A. L. HELLER. *This Journal* **141**: 518, 1944.
- (6) FREEMAN, N. E., J. L. SHAW AND J. C. SNYDER. *J. Clin. Investigation* **15**: 651, 1936.
- (7) ERLANGER, J., R. GESELL AND H. S. GASSER. *This Journal* **49**: 90, 1919.
- (8) CATTELL, MCK., JR. AND W. B. CANNON. *Arch. Surg.* **4**: 300, 1922.
- (9) BLALOCK, A. AND S. E. LEVY. *This Journal* **118**: 734, 1937.
- (10) WIGGERS, H. C. AND S. MIDDLETON. *This Journal* **140**: 677, 1943.
- (11) ZWEIFACH, B. W., R. E. LEE AND C. HYMAN. *Ann. Surg.* **120**: 232, 1944.

A STUDY OF THE EFFECT OF LIMITATION OF FOOD INTAKE AND THE METHOD OF FEEDING ON THE RATE OF WEIGHT GAIN DURING HYPOTHALAMIC OBESITY IN THE ALBINO RAT^{1, 2}.

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It has been known for many years that obesity may develop as a result of injury to the hypothalamus (7). Furthermore, obesity has been produced experimentally in various species of animals by making lesions in specific areas of this region of the brain (1, 2, 8, 13). Numerous attempts to determine the nature of the imbalance created by these obesity producing lesions (3, 11) have been made, but many questions still remain unanswered. The principal problem which presents itself in such a study is that of determining the cause of the failure of mechanisms which normally maintain the balance between food intake and energy utilization. Consequently, in the present investigation we attempted to detect some of the abnormalities which might contribute to the imbalance and evaluate their relative importance. Subsequent papers will deal with other aspects of this problem.

Obesity can be divided into two phases, a dynamic phase during which weight is gained at a rapid rate and a static phase during which there is no abnormal gain but the obese condition is maintained. In this latter phase apparently a balance is re-established between factors tending to increase and decrease the obesity. It seemed reasonable to assume, therefore, that the nature of the imbalance which causes the obesity could be detected most readily during the dynamic phase before readjustments occur which might abolish or mask the defects. The factors responsible for the termination of the dynamic phase of obesity and for the weight loss which eventually occurs as senescence develops must act in opposition to those which create the obese condition.

This experimentally produced obesity might be due to an increase in appetite, to a failure of the sense of satiety, to a change in the efficiency of the digestive system, to a decrease in energy output resulting from a lowered metabolism or a lowered level of activity without a corresponding decrease in food intake, or to a combination of these and other causative factors. Attempts have been made to determine whether or not these various possible contributory factors do operate. The portion of this study which is summarized in this paper deals with the rate of weight gain as affected by limitations of food intake and the method of feeding. While many of our experiments have produced data which merely amplify and support work previously done in other laboratories, it is thought that our studies

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have tested various hypotheses previously offered and also have contributed additional new information.

PROCEDURES. The animals used were white rats of a strain (Wistar derivative) which normally attains an adult weight of approximately 250 grams for the females and 350 grams for the males. They were kept in individual cages each of which was mounted over a large glass funnel to permit collection of urine. The diet consisted of Purina dog chow checkers or a mash made by mixing equal weights of water and dog chow. Once a week this regime was supplemented by a few grams of apple or sweet potato. No specific conditions of lighting or heat were maintained. The control animals, however, were subjected to conditions identical with those imposed upon the animals which became obese.

All animals were anesthetized with Evipal and lesions were made by means of a Horsley-Clarke stereotaxic instrument as described by Hetherington (8). In most cases the operations were performed on mature animals which had attained a rather stable body weight plateau. Thirty-five definitely obese rats and eighteen others which developed a low grade of obesity were studied. These were compared with normal litter mates and with other litter mates which failed to become obese because the hypothalamic lesions had not involved the critical areas. Additional details and procedures will be given as the various experiments are described.

RESULTS. 1. *Weight Gain on a Quantitatively Unlimited Diet.* Animals differed enormously in their rate of weight gain and food intake after operation. Presumably this was due to variations in the location of the lesions. There appeared to be a maximum obesity which was attained by a number of animals and figure 1 D portrays the development of obesity in one such case. Table 1 shows the increments in food intake and rates of weight gain in 15 experimental animals and 5 normal controls. In mature males and females weight gain was approximately 0.5 gram per day before operation. After effective operations the weight gain increased to between 3 and 8 grams per day, averaging 4.5 grams throughout the dynamic phase. As Brobeck, Tepperman and Long (3) have shown, a definite correlation exists between the amount of food intake and the rate of gain. The fact that rats gained much more weight per gram of food eaten after operation than before is probably chiefly due to the postoperative increase in appetite which induced the animals to ingest an excess of food, all of which excess was available for new tissue formation. Since complete balance studies were not made it was impossible to calculate how much food, over and above that required to meet energy outputs, was really available before and after operation for the formation of tissue components.

The dynamic phase of obesity persisted for one and a half to two months and by the end of that period body weight was approximately twice that at which it had practically stabilized before operation. It was concluded that the static phase had begun when the rapid weight gain ceased. This static phase may be defined as the period during which the animals neither lost nor gained weight or exhibited a rate of gain which did not exceed the preoperative rate. This phase was considered to have terminated when the animal began to lose weight slowly

and steadily, as they all did sooner or later when kept for many months. A few animals showed no definite static phase while in others it lasted for as long as 8 months during which time numerous fluctuations in level occurred (fig. 2-A-306). The life span of the majority of obese animals was much shorter than that of

TABLE 1

Food consumption and weight gain during normal phases of development and after obesity producing lesions had been made

OBESE FEMALES	NORMAL PERIOD				DYNAMIC PHASE				STATIC PHASE			
	Daily ave. for 30 days before operation				Daily ave. for 1st 30 days of dynamic phase				Daily ave. for 30 days of static phase			
	Wt. before op.	Body wt. gain	Food in-take	Wt. gain per gm. eaten	Body wt. gain	Food in-take	Wt. gain per gm. eaten	Duration of phase	Body wt. gain	Food in-take	Wt. gain per gm. eaten	Duration of phase
		gm.	gm.	gm.	gm.	gm.	gm.	days	gm.	gm.	gm.	days
No. 18.....	345	1.03	18.5	0.055	6.16	33.8	0.182	34	0.41	30.8	0.013	24
No. 19.....	283	0.50	18.7	0.026	4.11	29.7	0.138	73	0.59	23.2	0.025	49
No. 278.....	205	1.16	13.5	0.085	4.67	23.7	0.197	60	1.00	16.5	0.061	24+
No. 281.....	247	0.90	15.8	0.056	5.33	31.33	0.170	37	0	18.5	0	77
No. 284.....	266	1.03	16.6	0.062	3.33	29.6	0.112	56	0.62	25.3	0.024	74
No. 285.....	240	0.80	15.4	0.051	6.02	30.5	0.197	44	0	24.2	0	20+
No. 287.....	225	1.00	17.5	0.057	5.57	27.3	0.204	44	1.41	22.1	0.063	39+
No. 289.....	225	0.83	16.4	0.050	4.33	25.4	0.170	33	1.00	17.2	0.058	60
No. 290.....	220	1.00	15.4	0.064	5.16	30.2	0.170	39	0.20	19.0	0.010	30+
No. 291.....	203	0.37	12.6	0.029	3.56	26.6	0.134	53	1.17	20.9	0.055	58+
No. 302.....	290	0.67	15.0	0.044	3.17	24.9	0.127	32	1.00	20.3	0.049	35+
No. 304.....	250	0.23	12.6	0.018	2.00	22.1	0.090	38	0.10	17.4	0.005	29
No. 306.....	245	0.33	15.5	0.021	4.56	28.0	0.162	63	0.36	24.2	0.014	272+
No. 318.....	250	0.50	14.5	0.034	3.33	24.2	0.137	45	0.40	20.2	0.019	24
No. 332.....	260	0.33	12.3	0.026	6.16	26.2	0.235	57	0.06	21.0	0.028	20+
Averages.....	250	0.71	15.3	0.045	4.49	27.5	0.161	47	0.55	21.4	0.029	
NORMAL FEMALES												
No. 297.....	280	0	15.8	0	0.26	11.4	0.022	30	0	16.1	0	50+
No. 312.....	245	0.83	13.8	0.057	0.30	16.4	0.018	30	0.16	12.6	0.012	30+
No. 313.....	200	0.33	13.5	0.024	0.33	11.7	0.028	30	0.16	15.6	0.010	172+
No. 366.....	125	0.50	12.1	0.041	0.26	12.5	0.020	30	0.53	13.0	0.040	30+
No. 378.....	217	0.66	11.8	0.055	0.70	13.2	0.053	30	0.30	12.4	0.024	30+
Averages.....	213	0.46	13.4	0.035	0.37	13.0	0.028	30	0.23	13.9	0.017	

normal controls. This may have been due to an understandably greater susceptibility to heat and to skin infections induced by the animals' inability to care for their coats and by the production of pressure ulcers on feet, legs and ventral surfaces of the body. The effects of fat deposition on the function of the kidneys, liver and other organs may have been responsible for early death in some individuals. Any occurrence which hastened permanent deterioration of the animals

terminated the static phase of obesity. Those rats in which obesity was produced at an early age naturally tended to have longer static periods than did those which became obese late in life.

Figures 1-D and 2-C-D and table 1 indicate the magnitude of the change which occurred in quantity of food ingested. They illustrate the fact that growth curves paralleled variations in food intake. It can be seen that the largest daily intakes were not attained immediately after operation, but occurred after

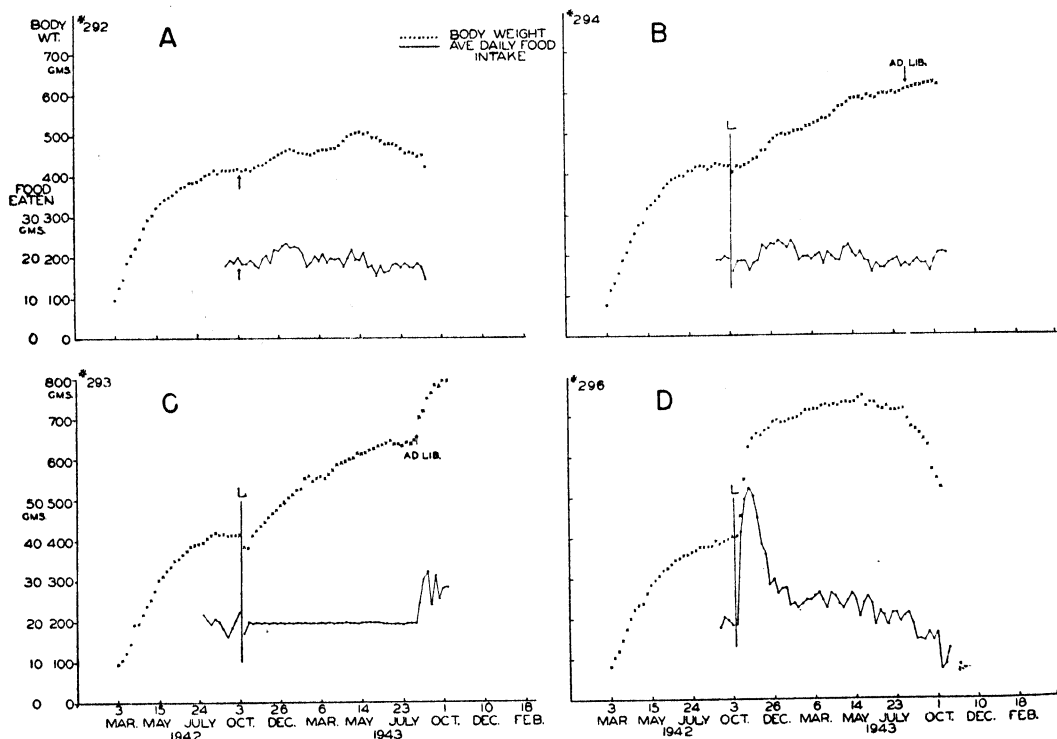


Fig. 1. A. Weight change and food intake of a normal rat. Arrows indicate time at which lesions were made in litter mates. B. Litter mate with lesion pair-fed with the normal. C. Litter mate with lesion limited to its preoperative average food intake. D. Litter mate with lesion fed ad libitum. *L* = hypothalamic lesions made. *AD. LIB.* = ad libitum feeding begun.

intervals of from one to three weeks. Such periods of time are required for the capacity of the gastro-intestinal tract to be sufficiently augmented to permit attainment of the peak intake. This maximal intake was not maintained for long; a decrease began even before the dynamic phase of obesity ended. After a few days the preliminary decline in quantitative intake ceased and during subsequent weeks the food consumption remained at a relatively constant value somewhat above the preoperative level of intake (fig. 1-D). This demonstrated that obesity can be maintained although the level of food intake is far below that which was present during the dynamic period of weight increment. The duration

of the static phase and the stability of body weight throughout this stage of obesity was determined by the constancy of food consumption levels. It is interesting to note that changes in body weight lagged considerably behind the declines in food consumption.

At the end of the static phase when animals became senescent both food intake and weight declined. It was observed in a few animals that body weight declined

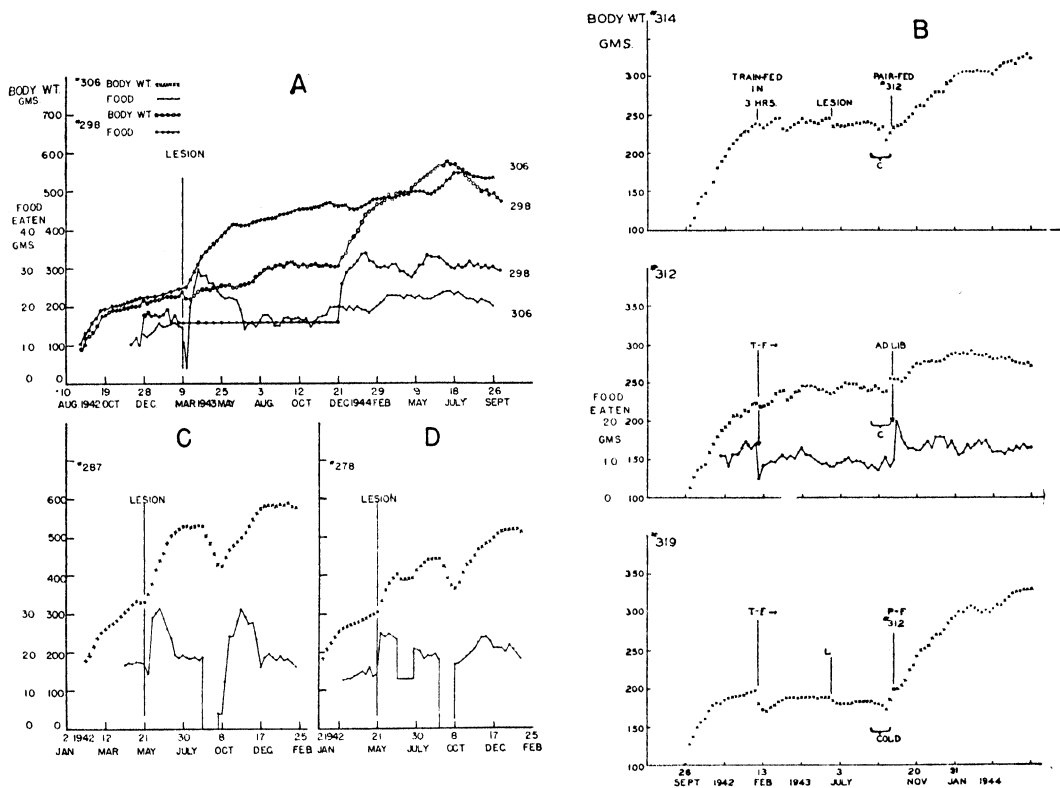


Fig. 2. A. Weight change and food intakes of rat 306 showing dynamic phase and fluctuations in a long continued static phase of obesity. Daily food intake and weight change in no. 298. This rat was limited to its preoperative intake which it received divided into 8 equal parts delivered at 3-hour intervals. B. Nos. 314 and 319 were pair-fed with the trained-fed control no. 312. After hypothalamic lesions were made nos. 314 and 319 failed to gain more than no. 312 until the control was fed ad libitum. C and D show weight changes during and after food limitation and starvation in obese animals.

markedly and even fell to the preoperative level even though the food consumption remained considerably above the normal level of intake until this fall was practically accomplished. This suggests an impaired use of food for weight production and maintenance. It is as if the animals' digestive systems were functioning less efficiently. There was no augmentation of activity or metabolic rate to produce a greater energy utilization.

2. Limitation of the Food Intake and the Development of Obesity. a. Duration

of limitation. The prevention of weight gain in the potentially obese animal by limitation of the food intake is possible. Formation of adipose tissue can be checked at any time provided sufficient limitations of caloric intake are imposed. The limitation must be severe and apparently it has no ameliorating effect on the appetite nor does it modify the tendency towards obesity when conditions permit. Evidence supporting this statement is given in figure 2-D which shows that obesity was checked in the dynamic phase by sudden restriction of the food intake. The amount of food given was slightly below the average preoperative consumption but despite this there was a gain of 12 grams during the 30 day period of limitation. In one case significant weight gain was prevented for 325 days by limitation of food intake. Food was then given ad libitum and the typical dynamic phase of obesity ensued. The animal consumed enormous amounts of food, gained weight rapidly and became maximally obese within the usual period of time. In a few other experiments of this type some animals which were thought to be potentially obese did not show hyperphagia and did not become obese after release from limitation. It has been observed, however, that some animals even when on an ad libitum diet suddenly lose their abnormal appetite and their weight plateaus only slightly above the preoperative level. It was thought, therefore, that limitation of the food intake had not been responsible for the disappearance of the hyperphagia. In many cases limitation of food or actual starvation did not abolish the tendency or drive towards obesity even though the hyperphagia was not permitted expression until very late in life and many months after hypothalamic injury. The significance of these few observations is that limitation must be severe and permanent to prevent obesity from developing following production of this type of hypothalamic lesion. The question then arises as to the degree of food limitation necessary to prevent the development of obesity.

b. *The effects of limiting food intake to the preoperative food consumption.* It was found that when potentially obese animals, rats with lesions properly placed to produce obesity, were limited to an amount of food which they habitually ate in the preoperative period they eventually became definitely obese although, of course, weight was not gained at as rapid a rate as in litter mates, which were fed ad libitum. These experiments were performed by determining the average daily food consumption of mature rats during one or two months prior to operation and then limiting their food intake to those amounts postoperatively. This was done in the case of ten rats selected from six litters the other individuals of which were either fed ad libitum or pair-fed (see below). In all these experiments the "self-limited" animals were unable to gain indefinitely or attain maximal obesity while limited in this way (fig. 1-C). It appeared that as more weight was added the energy used in essential activity increased and eventually overbalanced the processes initiated by the hypothalamic lesions which permitted the rats to deposit more fat than they had been storing on the same food intake before operation. After this plateau of intermediate obesity was established the food restrictions were removed; a burst of additional weight gain then occurred and maximal obesity soon developed.

Brobeck, Tepperman and Long (1943) pointed out that potentially obese rats have a voracious appetite after operation and tend to eat their entire day's ration within a few hours if the amount of food is at all limited. They suggested that this method of eating might influence an animal's ability to gain weight. Consequently, a device was built capable of delivering specific amounts of food at regular intervals (fig. 3). The influence of regularly spaced feeding on the development of obesity was determined in two litters of four rats each. Three animals of each litter were operated upon and one was retained as a control.

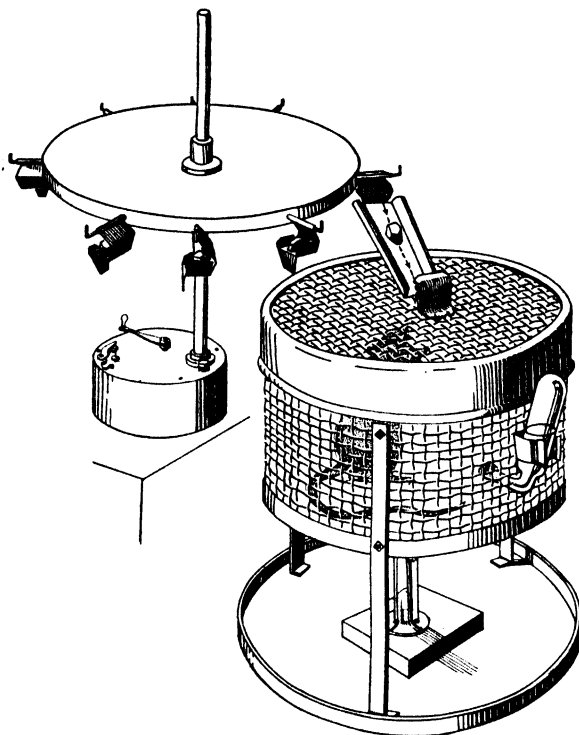


Fig. 3. A device used in delivering food at regular intervals in spaced-feeding experiments. A pendulum kymograph rotates the cup bearing wheel.

When temporary engorgement was prevented by subdividing the average daily preoperative food intake of each of these rats into eight equal parts which were delivered at three-hour intervals, it was found that four rats of the six with lesions outgained the two normal controls and became definitely obese (e.g., no. 298 of fig. 2-A-298). The two animals with lesions which failed to gain showed little response terminally when fed ad libitum while the four which had gained became maximally obese when they were released from the limitation.

In order to test the ability of animals with lesions to gain weight under conditions which had required compensatory adjustments to maintain their body weight during the period of normality, another slight modification of this "self-

comparison" type of experiment was performed. Tepperman, Brobeck and Long (3) demonstrated that at least one of the metabolic peculiarities of obese animals, a rise in R. Q. above unity in response to administration of glucose, could be produced in normal rats by training them to consume their daily ration of food within a brief period of time. Presumably this change is a sign of compensatory adjustments since it appeared as the animals regained an ability to maintain body weight at normal levels. In our experiment four female rats of one litter were trained to eat their daily ration of food within three hours. After a short period in which they lost weight they were able to regain and maintain their normal body weight and even to grow to some degree. Three of these animals were operated upon at the end of the second month and then were limited to an amount of food equal to the average daily intake of the training period. All ate their ration within the three hour period but none of them was able to gain significantly or keep up with the normal trained rat which was gaining weight because it was able to increase its three-hour intake as it grew. Since two of the three rats with lesions became obese when fed ad libitum it is clear that their lesions were effective. This experiment indicates that obesity-producing lesions do not enable animals to gain weight and deposit excess fat when placed on a feeding regime which compels normal animals to develop a compensatory response in order to maintain their body weight.

Certain conditions of these experiments tend to invalidate the use of the results in support of the contention that after hypothalamic injury a rat can become obese when it is limited to a "normal" food intake. For example, the "normal" food intake was the quantity of food eaten by the animal when it was younger and more active. Furthermore, since conditions of light and temperature were not controlled it is conceivable that these factors may have influenced the activity and metabolic needs of the animals in such a way as to have contributed to the postoperative gain in weight. Experiments to be described next eliminated these defects. It can be said, however, before proceeding that regardless of the cause of the decrease in energy output which makes possible a weight gain on a fixed energy intake, the primary defect created by the lesions was a breaking down of the normal relationship between appetite and energy need which maintains body weight within normal limits.

c. *The effects of pair-feeding.* A series of pair-feeding experiments was next performed. This second method of limiting the food intake to a "normal" level is preferable to the previously described procedures. Though no two animals are exactly alike this method of giving the animal with a lesion only an amount of food equivalent to that which its normal litter mate had just eaten eliminates fluctuations in requirement due to aging and environmental changes. Experiments of this type have been carried out by Hetherington and Ranson (11). They concluded that the potentially obese rats can become obese on the amount of food which their normal litter mates ingest. Brobeck, Tepperman and Long (3) performed a similar series of experiments under somewhat more stringent conditions; nevertheless, three out of twelve animals outgained the controls with which they were pair-fed. This work was repeated and we confirmed the obser-

vation that following the obesity-producing type of hypothalamic injury some animals do outgain their litter mate controls with which they are pair-fed. In no case was this gain as pronounced as in self-comparison experiments but it was unmistakable (fig. 1-B). In the case shown in this figure the potentially obese rat gained 0.80 gram per day as compared to 0.43 gram gained daily by the litter mate control.

Spaced feeding did not prevent animals which had been operated upon from outgaining spaced-fed normal litter mates. This experiment was performed with two litters in each of which three animals with lesions were pair-fed with one normal control. In only three of the animals with hypothalamic injuries were the lesions successful in the sense that hyperphagia remained permanently present and induced a well developed obesity when control of food intake was abolished. The weight surplus which these animals were able to accumulate in these pair-feeding, spaced-feeding experiments was in no case as great as the extra weight accumulated by the rats which were merely pair-fed. This difference is thought to be insignificant in view of the fact that the rate of gain of the various animals differed enormously.

In one other experiment two animals with effective lesions were compared with a normal control which was permitted access to food for only three hours daily. In order to maintain body weight under this regime compensation was necessary. The two litter mates were given only that amount of food which the control ate in three hours. Under these circumstances the two potential'ly obese rats did not gain more weight than the control. During a period of cold weather all animals were less able to maintain their body weight under the trained feeding regime. The lesion did not prevent the animals from compensating enough to restore body weight nor did they enable them to accumulate fat on an intake equivalent to the normal rats "trained-fed" intake. After 16 weeks the control was given free access to food at all times. The two animals, with lesions, which were still pair-fed with the control then outgained the control significantly and deposited excess fat (fig. 2B). These observations again indicate that the obesity-producing hypothalamic lesions confer upon some animals an ability to deposit more fat than normal rats which ingest the same amount of food but not if the normal rats are compelled to develop compensatory responses in order to maintain their body weights under conditions imposed.

d. *Some interesting consequences of starvation of obese animals were observed.* It has been shown many times that prolonged fasting will bring the weight of an obese animal down to a normal level. Brobeck, Tepperman and Long (3) published a figure in which it was shown that 2 fasting obese rats lost weight at a rate of between 7 and 10 grams per day. In our experiments 2 normal controls and 12 obese animals which had attained a static state of obesity were fasted for 28 days. The obese rats showed a maximum weight loss which occurred on the third day of starvation and averaged 16 grams. The average daily loss during the total period was 6.1 grams. This represented an average daily loss of 1.4 per cent of the initial weight. In the normal animals the maximum loss occurred on the first day and amounted to 8 grams. The average daily loss in weight was

2.6 grams or 1.2 per cent of the initial weight. This period of starvation did not reduce the obese animals to their preoperative weights but it did produce severe debilitation. Six of the starved rats refused to eat when food was first given and it was necessary to force feed them with vitamin rich foods for a few days before they began to eat spontaneously. The daily food consumption then rose rapidly to a level equal to that attained in the initial dynamic stage (fig. 2C and D). Rapid weight gain occurred but the rate of gain did not equal that of the initial dynamic phase of the obesity. It is difficult to judge whether weight loss was more or less rapid in starved obese rats than in starved normal controls. In the obese rat there is a higher percentage of stored material which can be lost. The obese animal is larger and necessarily uses more energy in essential activities. Consideration of these factors leads one to believe that the mechanism of weight loss is not essentially different in the two cases.

The interesting aspect of these experiments was that the new weight plateaus established in obese animals after periods of starvation were much higher than the initial levels of spontaneously attained weight balance (fig. 2C and D). This suggests that periods of ad libitum eating interspersed between periods of fasting may produce more intense obesity than that which develops when food intake is not interfered with.

3. *Loci of Effective Hypothalamic Lesions.* The loci of the lesions which produced obesity were determined in all animals. Although the position, extent and symmetry of the cerebral injuries varied considerably certain general statements can be made. Lesions which produced maximum obesity always destroyed or impinged upon the ventromedian nuclei bilaterally. The most effective lesions were the largest and they generally included portions of the nucleus dorsomedialis, premammillary area, medial and lateral mammillary nuclei or the tuberal region. Less effective lesions were bilaterally asymmetrical and usually failed to injure one ventromedian nucleus. In other instances lesions which produced only mild hyperphagia and obesity were merely less extensive. In several cases of intermediate obesity it would have been impossible to predict the degree of obesity from the boundaries of the injuries. Lesions which failed to produce obesity were located in the preoptic, lateral or supraoptic regions of the hypothalamus and the absence of obesity could have been predicted from inspection of the brains. These results are in entire agreement with those of Hetherington (9).

4. *Morphological Consequences of Hypothalamic Injury.* Table 2 gives certain body dimensions and weights and the weights of tissues and organs of some of the obese animals and their controls. Animals which were obese had markedly different girth-length ratio and nutritional index than did their normal controls. Abdominal fat deposits were greater and with the exception of the gonads and uteri the organ weights of these obese rats were equal to or greater than those of the controls. The weight increments of organs such as the heart, liver and kidneys were approximately 20 to 30 per cent above the normal and not of the same magnitude as the gains in total body weight which amounted to 200 to 300 per cent. It seems reasonable to assume that a degree of hypertrophy was required

of these organs to serve the demands of a larger body even though much of the excess weight was due to stored fat. Although the hypothalamic lesions did not impinge upon the hypophysis the small size of the gonads and reproductive organs suggests hypofunction of that gland. It has been reported on numerous occasions that hypothalamic lesions do affect reproductive functions (5, 10). It

TABLE 2

Average body size and the average weights of organs of 24 obese females and 6 normal controls

	BODY WT.	NOSE- ANUS LENGTH	GIRTH	GIRTH/ LENGTH	W. $\frac{1}{2}$ /L	TOTAL ABDOM. FAT	VENT. OF HEART	DIAPH.	SPLEEN
	gm.	cm.	cm.			gm.	gm.	gm.	gm.
Normal rats									
m.....	237	20.9	17.8	.852	0.293	11.6	0.96	1.07	1.08
σ	8.67	1.87	1.59	0.75	0.003	2.25	0.29	0.20	0.25
S.E.m.....	± 3.65	± 0.76	± 0.64	± 0.01	± 0.001	± 0.91	± 0.12	± 0.08	± 0.10
Obese rats									
m.....	443	20.6	24.2	1.17	0.367	63.6	1.13	1.02	1.04
σ	84.32	1.83	6.16	0.13	0.03	18.01	0.21	0.15	0.24
S.E.m.....	± 17.2	± 0.37	± 1.25	± 0.03	± 0.006	± 3.67	± 0.04	± 0.03	± 0.05
Per cent change									
m.....	+86.0	-0.01	+36.0	+37.0	+25.0	+448.0	+1.0	-4.0	-3.0

	LIVER	KIDNEYS		UTERUS	OVARIES		ADRENALS		THYROID	RT. SUB- MAX. GLAND
		R.	L.		R.	L.	R.	L.		
	gm.	gm.	gm.	gm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Normal rats										
m.....	8.8	1.06	1.06	0.95	34.5	31.7	34.6	34.4	16.9	203.1
σ	2.59	0.27	0.29	0.36	6.74	10.39	5.71	6.99	5.96	61.8
S.E.m.....	± 1.05	± 0.11	± 0.10	± 0.14	± 3.02	± 4.24	± 2.33	± 2.85	± 2.43	± 25.22
Obese rats										
m.....	13.2	1.27	1.26	0.75	16.7	18.7	37.9	38.2	22.7	213.2
σ	2.08	0.22	0.24	0.35	5.05	6.24	13.18	12.86	5.88	48.4
S.E.m.....	± 0.42	± 0.04	± 0.04	± 0.07	± 1.03	± 1.27	± 2.68	± 2.62	± 1.20	± 9.80
Per cent change										
m.....	+47.0	+18.0	+18.0	-21.0	-51.0	-41.0	+10.0	+1.1	+40.0	+5.0

is possible, however, that the hypogonadism might be secondary to the obesity. Despite the smaller size of the reproductive tissues estrous cycles were normally maintained in numerous obese animals. Five obese rats with normal cycles were placed periodically with males and mating did occur but pregnancy never resulted.

Histological examination of tissues and organs revealed no consistently present

abnormality which conceivably might have caused obesity. The kidneys of 7 of 35 rats studied contained scattered areas of degeneration similar to those described by Brobeck, Tepperman and Long (3). The testes of 6 of 10 males examined were degenerate and showed no signs of spermatogenesis. The ovaries of 5 of 19 females were clearly atrophic. In a few animals the pancreas showed much fatty infiltration and 20 livers out of a total of 34 studied were grossly abnormal in that they were loaded with fat. The adrenal glands of 23 of the 34 maximally obese rats which were studied contained cortical lesions varying considerably in magnitude. For the most part the lesions appeared to have originated from small hemorrhages in the basal layer of the cortical tissue. The cells of the follicular epithelium of the thyroid glands of the obese animals were very low and many of the follicles were enormously engorged with colloid. These histological changes and the larger size of the gland might be considered to be an indication of hypofunction. Metabolism tests to be reported later confirmed the supposition that the basal metabolic rates were lower in those animals whose thyroids showed these features indicative of hypofunction.

DISCUSSION. The fact that the body weight of mature normal animals is maintained at a rather constant level indicates that the food intake is nicely adjusted to balance changes in energy utilization. Lesions involving the region of the ventromedian nuclei of the hypothalamus destroy this balance. Hyperphagia occurs and enormous amounts of food are ingested if they are available. It likewise appears that associated with this augmentation of appetite there is a reduction in energy output. Since the appetite is not reduced, the animals tend to eat as much as normal rats with which they are pair-fed even though their energy output must be less since they gain more weight than do the normal controls.

Energy requirements do vary and it has been stated also that an animal's efficiency, judged by weight gain per calorie of food ingested (6), can change. The question of how hypothalamic lesions of the type described produce such an effect remains for future work to determine. It does seem certain that animals with effective lesions do have a greater ability than normal rats to store fat. This advantage conferred by the lesion is slight, however, and in none of our experiments did it enable animals with lesions to outgain normal animals which were forced by limitation of food intake to compensate in order to maintain body weight. It is apparent that in limiting food intake to prevent obesity one should use an animal's own energy utilization as an index of the minimum required rather than the food intake of a normal control or the animal's own preoperative intake.

Long prevention of obesity by limitation of food intake does not abolish the hyperphagia. As a matter of fact, periods of starvation in some cases seem to cause rats to attain a greater degree of obesity than that at which they had apparently stabilized before starvation. With senescence obesity tends to disappear at a rate which is not proportional to the decrease in food intake. The majority of obese rats with effective lesions die at the peak of their obesity of causes which cannot be due to aging.

As regards our studies of the site of effective lesions, of body and organ weights

and of the histological status of various organs and tissues, it can be said that our results confirm the observations of Hetherington and Ranson (12) and Brobeck, Tepperman and Long (3). The gonads are small and apparently somewhat hypofunctional. Other endocrine tissues and visceral organs are enlarged in obese animals and this may be indicative of an effort on their part to adequately serve a much larger mass of tissue.

SUMMARY

The rate of weight gain, changes in food consumption, the duration of the various phases of experimentally produced obesity and the loci of obesity producing lesions were determined in a series of albino rats.

Neither limitation of the daily food intake to that of the preoperative period nor pair-feeding with normal controls prevented development of a degree of obesity. This advantage conferred by the lesion is slight, however, and in none of our experiments did it enable animals with lesions to outgain normal animals which were forced by limitation of food intake to compensate metabolically in order to maintain body weight.

Prevention of temporary engorgement with food by spaced feedings did not prevent obesity but potentially obese animals were not able to outgain normal rats which had been forced to compensate metabolically in order to maintain their body weights under conditions imposed by feeding regimes.

Hyperphagia due to hypothalamic injury was not abolished by long continued limitation of food. Periods of starvation were followed by an augmentation of appetite and development of a greater degree of obesity than had been attained before fasting.

The major cause of this experimentally produced obesity was the breakdown of the balance between food intake and energy utilization. Inappropriate augmentation of the appetite was presumably accompanied by a less significant decrease in caloric requirement.

REFERENCES

- (1) ASCHNER, B. *Pflüger's Arch.* **146**: 1, 1942.
- (2) BAILEY, P. AND F. BREMER. *Arch. Int. Med.* **28**: 773, 1921.
- (3) BROBECK, J. R., J. TEPPERMAN AND C. N. H. LONG. *Yale J. Biol. and Med.* **15**: 831, 1943.
- (4) BROOKS, C. McC., E. F. LAMBERT AND P. BARD. *Fed. Proc.* **1**: 11, 1942.
- (5) BROOKS, C. McC. *Res. Publ. Assoc. Res. Nerv. Ment. Dis.* **20**: 525, 1940.
- (6) CAMPBELL, H. L. *This Journal* **143**: 428, 1945.
- (7) ERDHEIM, J. *Sitzungsber. d. k. Akad. d. Wissensch., Wein, Abt. III* **113**: 537, 1914.
- (8) HETHERINGTON, A. W. *Endocrinology* **26**: 264, 1940.
- (9) HETHERINGTON, A. W. *J. Comp. Neurol.* **80**: 33, 1944.
- (10) HETHERINGTON, A. W. AND S. W. RANSON. *Anat. Rec.* **78**: 149, 1940.
- (11) HETHERINGTON, A. W. AND S. W. RANSON. *This Journal* **136**: 609, 1942.
- (12) HETHERINGTON, A. W. AND S. W. RANSON. *J. Comp. Neurol.* **76**: 475, 1942.
- (13) SMITH, P. E. *J. A. M. A.* **88**: 158, 1927.

THE RELATIVE IMPORTANCE OF CHANGES IN ACTIVITY IN THE DEVELOPMENT OF EXPERIMENTALLY PRODUCED OBESITY IN THE RAT¹

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The adiposity which can be produced in rats by means of specially placed hypothalamic lesions (11, 21) must be the result of a food intake which is not limited by the energy requirements of these individuals. It is agreed generally that the immediate cause of the experimentally produced obesity is a tremendously increased food intake and, to a lesser degree, a possible concomitant decrease in spontaneous activity (7). Abundant evidence has been obtained to show that effective lesions do cause a marked hyperphagia but the question whether or not these lesions consistently produce sufficient reduction in total activity to affect significantly the energy output of the animals deserves further experimental exploration.

Numerous expressions connoting decreased levels of activity have been used in describing the characteristic behavior of animals which have become obese as a result of experimentally produced hypothalamic injuries. In some instances inactivity was apparent immediately after operation and could, therefore, conceivably be a contributory factor to the development of obesity rather than a mere result of extreme obesity. Hetherington and Ranson (12) actually measured the spontaneous running of rats before and after they had received hypothalamic lesions of the type which causes development of obesity. This report is merely a confirmation and elaboration of the experiments of Hetherington and Ranson.

METHODS. The spontaneous running activity of three control rats and seven animals which developed various degrees of obesity after operation was recorded. One type of apparatus used was practically identical with that described by Richter and Wang (18) and employed by Hetherington and Ranson (12). In approximately half of the cases the size of the "living" compartment was reduced to such a degree that it merely held the food cup and the graduated drinking device. In these instances the rats were forced to remain in the "running" wheel throughout the experiment.

Even when confined to the easily rotating wheel the rats made many movements which were not recorded. It appeared that a better estimation of the total activity might be made by using a cage mounted on tambours connected by means of an air system to a recording tambour (15). The tambour system was so constructed that very slight movements of the animals were sufficient to activate the system. Records of these movements were obtained by means of

¹ A preliminary report of this work was published in *Fed. Proc.* 5: 12, 1946.

slowly revolving smoked drums. The twenty-four hour records secured in this way enabled one to determine approximately the time, the frequency and the intensity of the activity. The records were analyzed by counting the number of excursions, which merely gave the frequency of the recorded movements, or by scanning the record with a photoelectric cell. This latter procedure consisted of holding the shellacked record between a strong light and the photoelectric cell and comparing the amount of light penetrating the dark background to the amount penetrating a similar adjacent area of the paper from which a portion of the soot had been removed by the excursions of the recording lever. This method though merely expressing activity in units of light intensity did record both the frequency and amplitude of the excursions of the recording device; it thus gave some measure of the intensity as well as the frequency of the activity. These results were suitable for purposes of comparison since the sensitivity of the recording tambours was kept relatively constant.

All experiments were carried on in a normally heated laboratory and no attempt was made to control the daily or seasonal fluctuations of temperature and light to which the rats were exposed. The control animals and those in which effective lesions had been made were subject to identical environmental conditions. All rats were fed between 9 and 10 a.m. each day and were given Purina dog chow pellets or, in some cases, a mash made by soaking these pellets in an equal weight of water.

RESULTS. Figures 1 and 2 present the results typically obtained by means of the revolving drum technique. During the normal preoperative period a four-day rhythm in running was recorded. Richter and his associates (15) have demonstrated that this activity cycle coincides with the estrous cycle and is caused by fluctuations in hormone level. In all animals studied, production of the hypothalamic lesions caused at least a temporary disturbance of the estrous cycle and an associated loss of fluctuations in the amount of running. In the case shown in figure 1 and in two other rats, control lesions were made. These lesions produced no significant change in appetite or body weight but they did cause a lengthening of the estrous cycles in two animals and a permanent loss of cycles in the third rat. In these three animals activity was markedly reduced for sufficiently long periods to have enabled obesity to develop. Since no significant gain in body weight occurred these experiments prove that it is possible to reduce running activity by means of hypothalamic injury without creating obesity.

The latter part of figure 1 and figure 2 confirm the observation made by Hetherington and Ranson (12) that obesity-producing lesions cause a marked decrease in running activity. In most instances there was a period of hyperactivity which lasted for 12 to 48 hours after operation. This was followed by a somewhat longer period of quiescence in which practically no running occurred. The animals gradually became more active and many showed slight regular or irregular increments in running associated with the regular or irregular occurrence of estrous cycles. In no case observed did the activity of a potentially obese animal return to normal levels of intensity. As the rats became obese, running in the

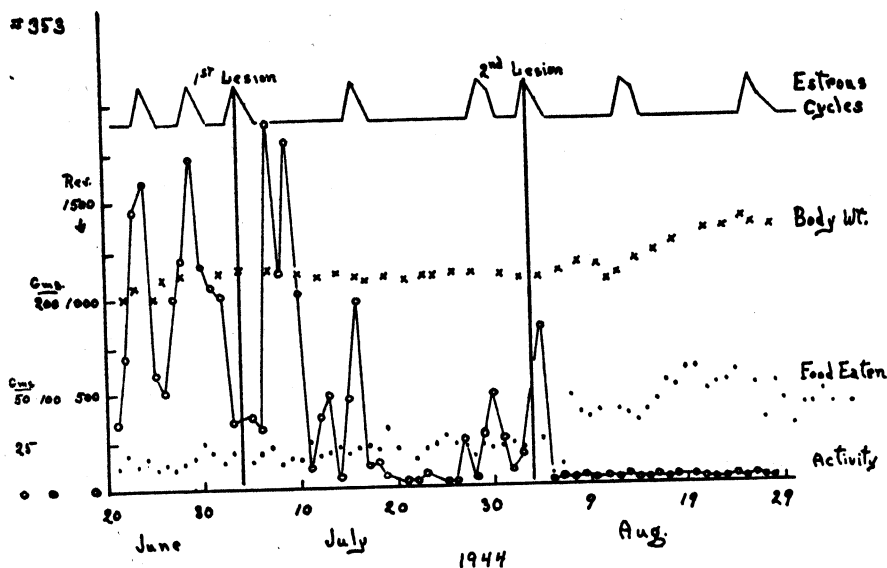


Fig. 1. Body weight, food intake, activity changes and modifications of the estrous cycle induced by hypothalamic lesions. The 1st lesion produced a reduction in activity and a modification of estrous rhythm but no hyperphagia or obesity. The second lesion produced a further reduction in activity and a mild degree of obesity.

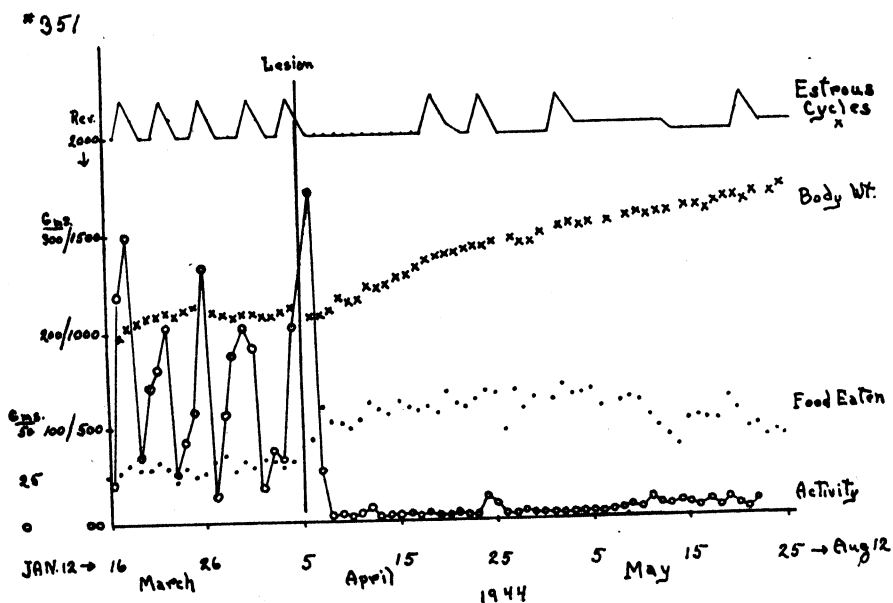


Fig. 2. Abolition of running activity and the development of obesity after operation.

wheel gradually became more difficult and finally impossible because of the fat deposits about their legs, chests and abdomens. In one case there was no period of postoperative hyperactivity. This animal became abnormally placid and sleepy. The other six rats showed the hyperirritability characteristic of many rats which became obese as a result of hypothalamic injury. The degree of obesity obtained varied considerably but in all cases the reduction in running activity was very great and persisted until termination of the experiments. There appeared to be no correlation between the degree of reduction in activity and the speed of development or degree of obesity attained.

Experiments performed with tambour-supported cages likewise revealed the estrous cycle variations in activity. The records also showed that throughout the day, periods of heightened activity appeared at regular intervals. This rhythm has been described and discussed by Richter (15) and others (20). During the hour between 9 and 10 a.m. when cages were cleaned and fresh food and water given, the rats were very active presumably because of the numerous extraneous stimuli. With this exception the normal rats were much less active during the daylight hours (6 a.m. to 6 p.m.) than at night (6 p.m. to 6 a.m.) (14). At night periods of activity were more frequent, more intense and of longer duration. In all normal rats studied night activity clearly exceeded day activity. A difference of 24 per cent was the minimum and 51 per cent was the maximum recorded. Lesions which did not produce hyperphagia or obesity did not modify this relationship but in the rats which eventually became obese the daylight activity became equal to or slightly exceeded the night activity. It was felt that this change in emphasis of activity was due to the fact that the eating habits changed. The potentially obese animals ate during the day as frequently as during the night (6) and this tendency apparently dominated other motivating influences.

The tambour recording method supported certain conclusions suggested by variations in running activity. Again it was found in four cases that certain lesions produced a reduction in activity but no significant gain in body weight. Figures 3 and 4 portray results typical of those obtained in seven rats which became obese. They show the brief postoperative period of hyperactivity which is characteristically followed by almost complete quiescence. The method, however, revealed one error which might be made if one based all conclusions on measurements of running activity. It can be seen in figure 3 that the total activity of the potentially obese animal gradually increased as the rat gained in weight. The activity remained subnormal for several weeks and this may have facilitated weight gain during the dynamic stage of obesity. However, the activity finally exceeded the preoperative level even while weight gain continued. These experiments again prove beyond question that a reduction in activity cannot be the sole cause of the experimentally produced obesity.

As the animals become more obese and progressively heavier the amount of energy required for the execution of movements essential to eating, shifting position, washing, etc., gradually increases. It was observed (see fig. 3) that within a short time after the recorded activity exceeded the preoperative level

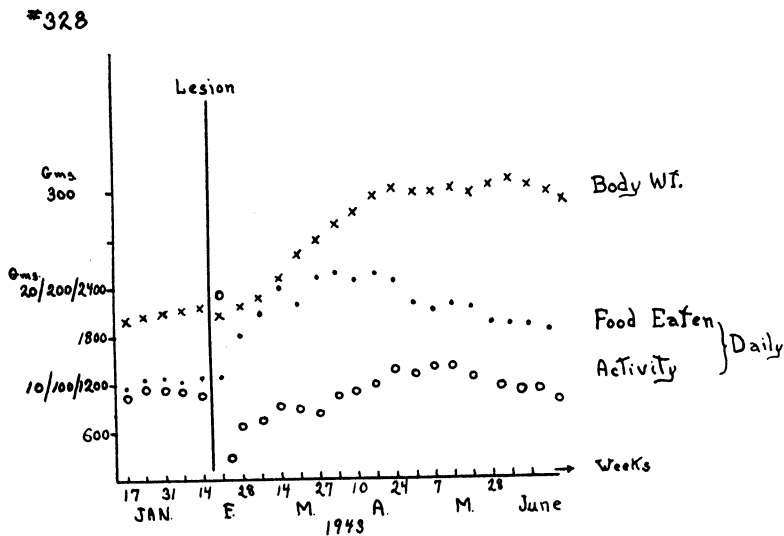


Fig. 3. Changes in tambour-recorded activity, food intake and body weight after hypothalamic injury. This shows the rise of total recorded activity above the normal level at the beginning of the static phase of obesity.

FREQUENCY OF MOVEMENT

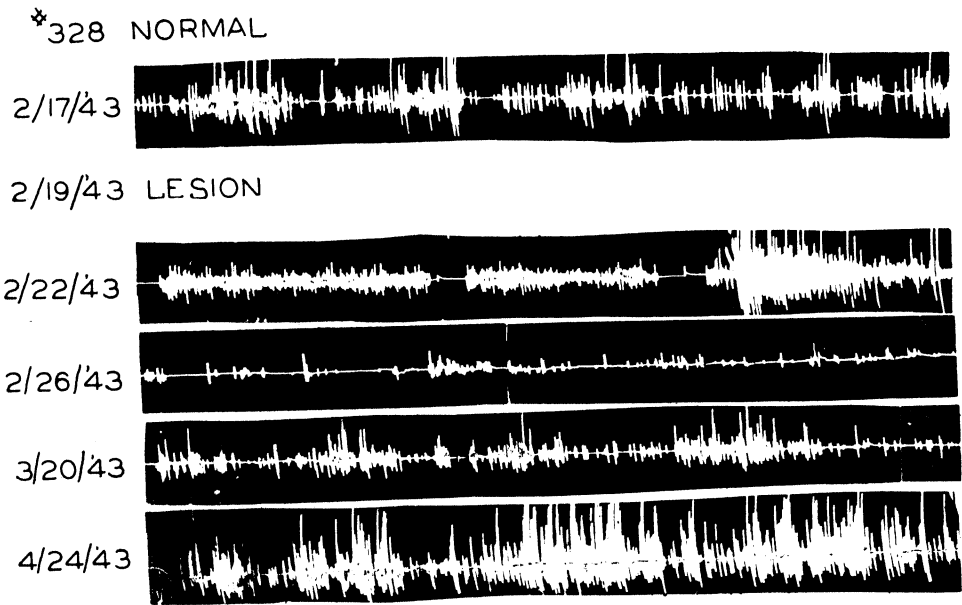


Fig. 4. Records obtained from the tambour activity recorded before and at various times after operation. They show various rhythms of activity. These segments of records are all for the same period of the day (1 p.m. to 10 p.m.). Same animal as that of figure 3.

the animals attained the static phase of obesity and body weight plateaued. Although running would have been practically impossible the tambours revealed a greater total activity which certainly must have contributed to the restoration of energy balance and thus facilitated the establishment of the static phase of obesity. This suggests that the increase in energy required for essential activity is a factor in limiting weight gain and in re-establishment of energy input-output balance. At any rate, body weight, food consumption and activity reach a peak and plateau.

Within a few weeks after the static phase of obesity is attained food consumption generally begins to decline (1, 4) but the body weight tends to remain stable for a much longer time. The fact that the recorded activity decreases somewhat shortly after food intake drops off may partially explain the maintenance of body weight at a time when energy intake is diminishing. As senescence develops obese animals tend to suffer from foot and skin lesions which may contribute to the decline in activity, food intake and body weight which occur terminally.

The pre- and postoperative activity of two animals which were limited to their preoperative food intake was measured. Both rats showed a reduction in total recorded activity after the hypothalamic lesions had been made. Both attained a moderate degree of obesity before given food ad libidum. During the period of limited food intake and weight gain, activity remained subnormal but gradually increased until it fluctuated about a level only slightly below the preoperation standard. When weight and activity appeared to be plateaued the ration was reduced to a level which was 10 per cent below their preoperative intake. The activity decreased as one might expect it to in starvation (14) but this compensatory change in requirement did not enable the animals to maintain their body weight. They were then given unlimited food. Body weight increased rapidly and the total measured activity immediately rose above the normal level. One of the rats was given its food in divided portions which were mechanically delivered at regular intervals (4). Each time the conveyor approached the delivery chute the animal showed excitement. Its activity exceeded that of the animal which was fed but once a day and its weight gain was not as great. Nevertheless, its total activity was less than it had been before operation. The reduction in activity may have been in part responsible for the weight gain which occurred on the limited diet but other factors should be considered (3, 5). The relationship between weight change and activity has been studied recently by Brobeck (2).

Lesions which caused a reduction in activity but no obesity varied somewhat in location and insufficient numbers of animals were studied to permit definite localization of the effective injury to any specific nuclear group or fiber pathway. In rat 353 of figure 1 the primary lesions were caudal and lateral to the ventromedian area. Those lesions which were followed by the development of obesity were located in the region of the ventromedian nuclei (4). Hetherington's papers on obesity contain more elaborate descriptions of the loci of effective and ineffective injuries.

Activity may be linked with the control of emotions, sleep and responsiveness to various stimuli. Five rats which became obese but were not used in activity recordings were obviously inactive and sleepy after operation. The lesions in these individuals were large and involved the mammillary and posterior lateral hypothalamic regions. Lesions which produced obesity in association with hyperirritability were more rostrally located. No analysis of the rôles of various hypothalamic nuclei in the control of emotional and other activity in the rat will be possible until further work is done.

DISCUSSION. No method of measuring accurately the total activity of an animal during long periods of time is available. Therefore, it is impossible to determine the fraction of the total energy output due to activity. The frequently employed procedure of estimating activity by recording running is inadequate for determining the significance of reductions in activity in the development of obesity. Records of the movements of rats in tambour-supported cages are not completely satisfactory criteria of total activity but they do show that animals which do not or cannot run may engage in activities which require a greater expenditure of energy than was expended in activity before operation. It is obvious that the tambours may be unaffected by many movements made by a rat when normal and small but when the same rat weighs 500 to 800 grams these same body movements produce marked excursions of the recorder. The energy used in executing the recorded movements must be proportionately greater. The defects of such a recording system are numerous. Beyond a certain frequency or speed of movement the system cannot record the separate elements of activity and beyond a certain point the relationship between the excursions produced by small movements and those produced by the grosser movements, involving greater weight shifts, are not linear. Despite these defects of recording one can conclude that obesity producing lesions do cause a reduction in total activity which may give the animal some advantage over the normal in accumulating fat deposits. That this is not the sole cause of obesity is amply shown by the fact that similar reductions in activity can be produced without causing obesity although a slight weight gain generally occurs. This conclusion is also supported by the fact that the energy expended in activity exceeds the preoperative level before weight gain ceases in the dynamic phase of obesity.

There are various possible explanations of the reduction in activity caused by hypothalamic lesions. All the hypothalamic lesions produced were in the proximity of the hypothalamico-hypophysial connections and might have interfered with hypophysial functions, thus impairing the activities of one or more of the dependent endocrine glands. It is known that removal of ovaries, testes, thyroid, adrenals or hypophysis does cause a decrease in level of activity (8, 13, 15, 16, 17, 19, 22). The degree of inactivity produced by hypothalamic lesions is of the same magnitude as that resulting from experimentally produced glandular deficiencies. In the majority of animals studied histological evidence of glandular dysfunction was found. In some females which developed adiposity the absence or irregularity of estrous cycles indicated a hypofunctional state of

the ovaries and hypophysis but in a considerable number of cases the cycles were normal and the histological studies revealed no evidence of subnormality. Underactivity of the endocrines due to absence or hypofunction of the hypophysis cannot in itself cause obesity since hypophysectomized rats do not become obese unless they are subjected to specific hypothalamic lesions (10). Some of the animals studied were obviously unresponsive to stimuli which produced activity in normal rats. These few animals appeared to sleep much of the time. Hypothalamic lesions can produce degrees of somnolence (9), but in the majority of animals studied in this series of experiments there was no obvious hypersomnia. It is conceivable also that lesions such as these which do produce changes in metabolism, temperature regulation etc., may indirectly modify general activity through these defects but no effort was made to ascertain the underlying causes of the inactivity observed. This underactivity may have had quite different bases in the several animals studied.

A reduction in activity can lead to excess fat deposition only in those animals which retain a normal appetite or acquire some degree of hyperphagia. Therefore, it is felt that the abnormal control of hunger or appetite is the primary factor in the production of hypothalamic obesity. The reduction in activity, at most, is merely a contributory factor. In combination with a lowered basal energy requirement (5) reduced activity may explain the fact that a potentially obese rat frequently outgains a normal rat when both are given the same amount of food (4).

SUMMARY

Normal rats are more active at night than during the day. Rats which tend to become obese following the experimental production of hypothalamic lesions lose this characteristic. Night and day activities become practically quantitatively identical.

A 12 to 48 hour period of intense activity usually occurs immediately after operation. This is in turn followed by a period of quiescence which lasts for a slightly longer period of time. In some instances the transient hyperactivity does not precede the period of reduced activity.

Postoperative activity gradually increases in frequency and intensity. The greater weight of the animal requires more energy for movement and the weight shifts involved in such activity as eating, washing, etc. become significant. The recorded activity finally exceeds the preoperative activity of the individual even before weight gain has ceased.

It is felt that the increase in energy expenditure involved in the essential body movements made by these fat rats is sufficient to contribute to the reestablishment of energy equilibrium and to the plateauing of the body weight.

After establishment of the static phase of obesity the food intake decreases. Body weight does not decrease to a corresponding degree because the activity likewise decreases. As senescence begins the food intake continues to decline and body weight begins to decrease even though the activity continues to decline.

In those animals in which the food intake is limited to their preoperative intake level slight but significant gains in weight do occur after operation. This has been attributed in part to an advantage conferred on the potentially obese animal by the reduction in activity. If the food intake is reduced much below the preoperative level the animals cannot gain weight.

It is agreed that the increase in appetite which occurs following the production of specific hypothalamic lesions, is the primary factor in the production of obesity, but it is felt that the reduction in spontaneous activity is sufficient to contribute to the slight weight-gaining advantage these animals possess when compared to the normal rat.

The lesions which produced obesity and the degree of inactivity described involved the region of the ventromedian nuclei of the hypothalamus.

REFERENCES

- (1) BROBECK, J. R., J. TEPPERMAN AND C. N. H. LONG. *Yale J. Biol. and Med.* **15**: 831, 1943.
- (2) BROBECK, J. R. *This Journal* **143**: 1, 1945.
- (3) BROOKS, C. McC. *Fed. Proc.* **4**: 9, 1945.
- (4) BROOKS, C. McC. AND E. F. LAMBERT. *This Journal* **147**: 695, 1946.
- (5) BROOKS, C. McC., D. N. MARINE AND E. F. LAMBERT. *This Journal* **147**: 717, 1946.
- (6) BROOKS, C. McC., R. A. LOCKWOOD AND M. L. WIGGINS. *Fed. Proc.* **4**: 9, 1945.
- (7) CONN, J. W. *Physiol. Rev.* **24**: 31, 1944.
- (8) HALL, V. E. AND M. LINDSAY. *Endocrinology* **22**: 66, 1938.
- (9) HARRISON, F. *Res. Publ. Assoc. Res. Nerv. and Ment. Dis.* **20**: 635, 1940.
- (10) HETHERINGTON, A. W. *This Journal* **140**: 89, 1943.
- (11) HETHERINGTON, A. W. AND S. W. RANSON. *Anat. Rec.* **78**: 149, 1940.
- (12) HETHERINGTON, A. W. AND S. W. RANSON. *This Journal* **136**: 609, 1942.
- (13) HUNT, J. McV. AND H. SCHLOSSBERG. *J. Comp. Psychol.* **28**: 23, 1939.
- (14) RICHTER, C. P. *Comp. Psychol. Mono.* **1**: 1, 1922.
- (15) RICHTER, C. P. *Quart. Rev. Biol.* **2**: 307, 1927.
- (16) RICHTER, C. P. *Endocrinology* **17**: 73, 445, 1933.
- (17) RICHTER, C. P. AND J. F. ECKERT. *Endocrinology* **21**: 481, 1937.
- (18) RICHTER, C. P. AND G. H. WANG. *J. Lab. and Clin. Med.* **12**: 289, 1926.
- (19) RICHTER, C. P. AND G. B. WISLOCKI. *This Journal* **86**: 651, 1928.
- (20) SKINNER, B. F. *J. Gen. Psychol.* **9**: 3, 1933.
- (21) TEPPERMAN, J., J. R. BROBECK AND C. N. H. LONG. *This Journal* **133**: 468, 1941.
- (22) WANG, G. H. *Comp. Psychol. Mono.* **2**: 1, 1923.

A STUDY OF THE FOOD-FECES RATIOS AND OF THE OXYGEN CONSUMPTION OF ALBINO RATS DURING VARIOUS PHASES OF EXPERIMENTALLY PRODUCED OBESITY^{1, 2}

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Numerous experiments have demonstrated that hyperphagia rather than a change in basal metabolic rate is the primary cause of the obesity resulting from experimentally produced hypothalamic lesions (1, 2, 9). There is evidence, however, to support the hypothesis that a reduction in energy requirement may contribute to the obesity. For example, it has been shown that after the production of appropriately placed hypothalamic lesions animals can attain a degree of obesity even when limited to their preoperative food intake (2) or when paired with normal rats (1, 2). We endeavored to test this hypothesis by determining whether there was a lower basal energy utilization in animals rendered potentially obese by appropriately placed hypothalamic lesions. We were interested, likewise, in determining the ability of obese animals to respond with increases in metabolism under conditions which would induce normal rats to show an augmentation of energy utilization. Finally, we determined the effect of abnormally high metabolic rates on weight gain during the dynamic phase of obesity. The food intake-feces output ratios of all these animals were likewise determined throughout the duration of these experiments. It is conceivable that a change in the ability of the digestive system to remove usable material from ingested food could contribute to body weight gain.

It was felt that the investigation of experimentally produced obesity might reveal facts not readily seen in clinical material. This type of obesity can be produced almost at will, consequently conditions during all phases of its development can be studied. It seemed reasonable to assume that any imbalance between energy intake and output which might occur in obesity could be detected more readily during the dynamic phase of weight gain than during the static phase of weight maintenance. These experiments were performed with this thought in mind.

METHODS. The oxygen usage of eighteen female rats which were members of four litters was determined once or twice weekly for several months before obesity-producing hypothalamic lesions were made in fourteen of them. Determinations of basal metabolism were continued for many months after operation until symptoms of senescence developed.

The rats were anesthetized with Evipal and brain lesions were made, as described by Hetherington (6), by means of a Horsley-Clarke stereotaxic instru-

¹ A preliminary report of this work was published in the *Fed. Proc.* 5: 12, 1946.

² The apparatus used for measuring the rate of oxygen consumption was designed and built by Dr. John D. Elder, Jr.

ment. The apparatus employed in the determination of oxygen use was a closed circuit device with a bellows pump which had an output of 110 cc. per minute. The air was passed over tubes filled with soda lime and calcium chloride filled tubes to remove carbon dioxide and excess water. The maximum pressure within the system did not exceed 10 cm. of water above atmospheric. The rate of use of oxygen was determined by measuring the time required for the disappearance of a given amount of oxygen from the storage reservoirs (100 cc. burettes) which were filled and emptied in alternation as the animals withdrew oxygen from the system. On each test day four or more separate determinations were made of the rate of oxygen usage by each animal. Unless they were obviously defective these tests were averaged and the results expressed as one figure. The carbon dioxide output was determined by weighing the soda lime tubes after a test interval. After a few weeks all the rats became so well trained that they lay quietly or slept during the tests.

The metabolic rate was raised above normal in some cases by giving thyroxin with the food. All animals were fed a mash consisting of Purina dog chow pellets mixed with an equal weight of water. Food intake was determined by daily weighing the dry residue. The dry weight of the feces, the water drunk and the urine output were likewise determined.

Toward the end of the experiments all rats except some normal and obese individuals which were kept as controls were placed in cages in a cold room for a period of one week to two weeks. The temperature of the room varied between 0°C. and 4°C. throughout 24 hours. Rectal temperatures were taken before entrance and at regular intervals throughout the first 6 to 8 hours. Thereafter the temperatures were taken once or twice daily during 7 to 12 days. For several hours immediately after removal of the rats from the cold room temperatures were again taken at short intervals. At autopsy all tissues of interest in this connection were weighed, fixed and prepared for histological study. Tissue weights and body size statistics of these rats were included in the tables presented in a previous paper (2).

RESULTS. 1. *The efficiency of digestive functions as indicated by food-feces ratios.* In ad libitum fed obese rats the feces output doubled or tripled as did the food intake immediately after operation. The ratio between dry weight of food eaten and dry weight of feces changed when the lesions were made. The feces output represented a higher percentage of the food intake. This suggested a lessened efficiency of the digestive system. In two series of rats (6 obese and 2 controls) in which the food intake was limited to that of the normals the feces-food ratios were not significantly different. In one series the dry weight of the normal rat's feces averaged 24.4 per cent of the dry weight of the food ingested. In the three animals pair-fed with the normal the percentages were 18.4, 21.6 and 21.8. In the second series the animals were fed at spaced intervals (2) as well as pair-fed with the control. The weight of the feces of the control was equivalent to 22.5 per cent of the dry weight of the food while the percentages in the three animals with lesions were 22.4, 22.2 and 16.6.

Even if one assumes that these differences in ratios did indicate a more efficient

action of the digestive system in the obese rats the change was insignificant. The maximum weight excess which could have been gained in this way was, in the first series, 24 grams, 13 grams and 10.8 grams during a 20 week period, and in the second, which was studied for a shorter period, 0.6 gram, 2.0 grams and 4.5 grams. Furthermore, the animal with the most favorable ratio for weight gain actually gained the least weight in terms of percentage of initial weight. It is felt that the differences were too slight to signify a change in digestive efficiency and the maximal observed change in food-feces ratio would have been too slight to account for the postoperative development of obesity. The fact that such a change in ratio was not present in the *ad libitum* fed rats likewise detracts from its significance. These determinations merely confirm the accepted concept (8) that obesity cannot be due to an increased efficiency of the digestive mechanism. They do not eliminate, however, the possibility of selective changes in digestion and absorption.

2. *Oxygen utilization in ad libitum fed animals.* The oxygen usage of four litter mates was determined at regular intervals throughout a three month period during which the rats attained maturity. Three of the animals were then operated upon and their oxygen usage compared with that of the normal control as they progressively became more obese. Figure 1 summarizes the results obtained and it can be seen that the total oxygen use of two of the rats remained below that of the control during the dynamic phase of obesity. The oxygen consumption surpassed that of the normal rat at a time when weight gain ceased. This may have been a factor in establishment of the static phase of obesity. The third rat (no. 382) which gained most rapidly did not show a subnormal total oxygen use but the animal was considerably larger than the control before operation and the weight discrepancy increased rapidly. In all 3 animals with hypothalamic lesions the oxygen use per unit of body weight or surface area was below that of the preoperative period and below that of the control. These results, though not completely convincing, did suggest that a lowering of basal energy requirement had occurred after operation and that it might have contributed to the development of obesity.

In those cases and during those intervals in which total postoperative oxygen use remained below normal the existence of a lower basal rate could not be questioned but in cases in which it was necessary to relate oxygen use to unit of tissue weight or surface area in order to show the reduction, the evidence was less convincing. Talbot (11) has shown that in children the oxygen use per unit of weight decreases rather precipitously during the period of rapid growth. Kibler and Brody (7) found in studies of young rats that after four months there was a gradual decrease in metabolic rate with increasing weight and age. In our studies we found in a series of 12 normal rats, in which oxygen use per unit of body weight was determined throughout the period of growth, that the oxygen consumption did decrease for several weeks as the animals gained weight. Similar calculations made on twelve rats which were becoming obese showed a similar reduction in oxygen use per unit of surface area or weight as the animals increased in size. This change in the obese animals was much less, however,

than that observed during the phases of normal growth. Regardless of what the explanation of this phenomenon may be it indicates that in comparing the basal oxygen use of normal and obese rats one should use animals of approximately the same size. In order to make such comparisons normal litter mate controls of the same age could not be compared with the obese animals but it was possible to compare the obese rats with older normal individuals of other litters. Large

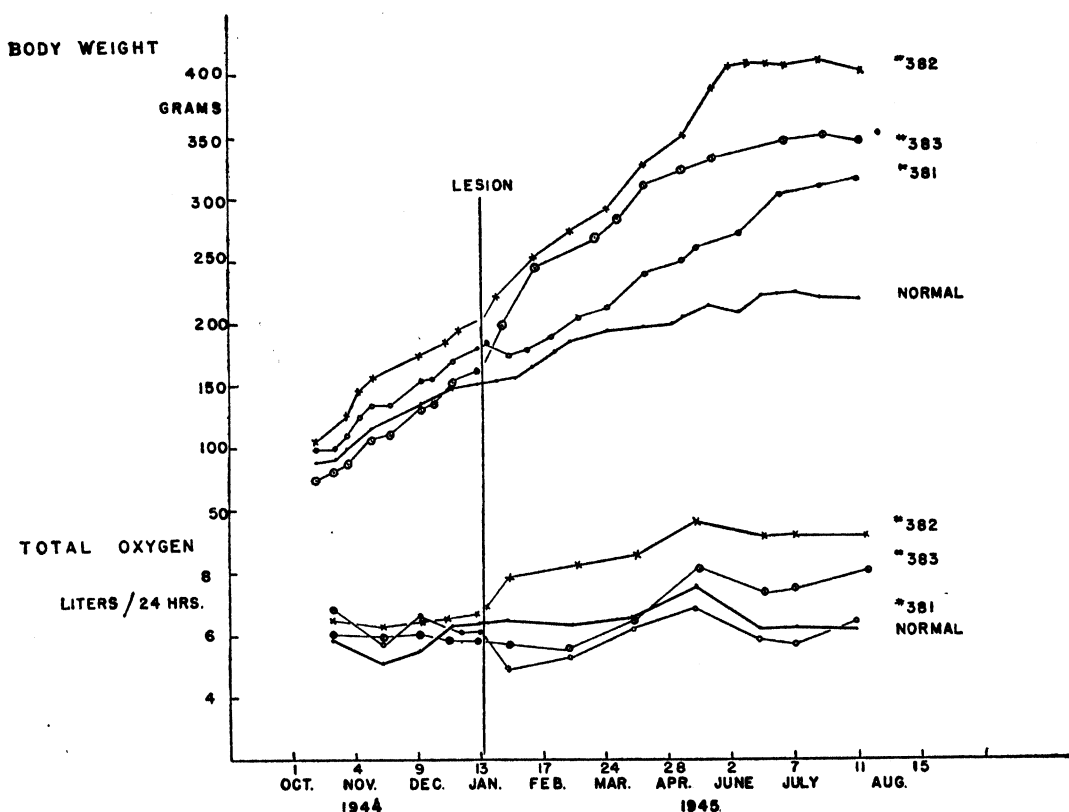


Fig. 1. The average daily oxygen consumption and weight curves of one normal rat and 3 litter mates with obesity-producing hypothalamic lesions. Each point on the curves represents the average of all determinations of oxygen consumption or body weight made between the date indicated and the date of the previous point.

old normal rats were compared with younger obese rats until the obese animals had attained weights never equalled by normals. Such comparisons were made between the 12 normal females which had been tested throughout their life span and 12 obese females in which similar measurements had been made. It was found that these obese animals practically always used less oxygen per unit than did normal animals of the same weight. In the 38 comparisons made between a normal and an obese animal there were only 3 exceptions to this rule. Furthermore, only 4 of the series of 12 normal females previously mentioned attained a peak of oxygen use of 8 liters per day. They weighed 230, 235, 242

and 243 grams respectively at the time of maximal oxygen consumption. Only 6 of the 12 obese rats which were compared with them attained a total basal consumption of 8 liters or more per day but these animals weighed 285, 330, 335, 345, 350, 390 grams at that time. These random comparisons of obese animals with normal rats of similar weights tended to confirm the observation that animals becoming obese following hypothalamic injury do have a slightly lower basal oxygen requirement.

3. *Oxygen utilization in potentially obese animals with a limited food intake.* It was thought that the effects of hypothalamic lesions on oxygen use might be masked less by changes in body weight if the development of obesity were inhibited by limitation of the food intake. Figure 2 gives the results of one such experiment. Animal 394 was prevented from exceeding its litter mate control in body weight for 3 months by limiting its food intake. During that interval both the total oxygen use and the oxygen use per unit of body weight were significantly lower than that of the normal rat. When food was given ad libitum this animal consumed more food than did the normal and soon attained a much greater body weight. The total oxygen consumption rose to equal and then exceed that of the control but the oxygen use per unit of body weight remained below normal. Two other similar experiments gave the same result and they support the hypothesis that hypothalamic lesions reduce oxygen consumption and thus, if food intake is not reduced, do confer a weight gaining advantage which may be detectable under certain circumstances. This reduction in oxygen utilization helps explain the ability of some animals with lesions to outgain normal animals with which they are pair-fed or to gain weight when restricted to their own preoperative intake. In one series of 4 rats the 3 which had hypothalamic lesions slowly outgained the normal litter mate with which they were pair-fed and showed lower basal oxygen consumptions. The magnitude of this lowered metabolic requirement conferred by hypothalamic lesions, as expressed in percentages, is shown in table 1.

Animal 392 (see fig. 2 and table 1) showed a second interesting occurrence. The hypothalamic lesion reduced the basal oxygen consumption as much as in rat 394 which became slightly obese but the animal (no. 392) did not become obese. The explanation of this apparently lies in the fact that its appetite decreased appropriately and its caloric intake diminished and remained in balance with caloric output. This confirms the contention (1, 2) that the major defect is the breakdown of the proper relationship between food intake and energy output. A decreased basal metabolic requirement cannot contribute to the development of adiposity unless accompanied by a degree of hyperphagia.

4. *Weight gain in obesity when a reduction in O₂ use is prevented.* That the reduction in basal energy requirement which results from hypothalamic injury is of relatively minor importance in the development of obesity has been shown in another type of experiment. To two individuals of one litter and one rat of a second litter sufficient thyroxin was given to prevent the postoperative reduction in oxygen consumption. As shown in figure 3, which gives the records of animals in the first litter, there was a slight reduction in rate of weight gain. This

indicates that the food intake was sufficient to overbalance an increase in requirement when sufficient food was available. In these experiments food consumption was not augmented during the period of thyroxin administration. Thiouracil given to one obese rat of this series did not reduce the basal oxygen consumption below that of other rats but there was no obvious influence on the rate of weight gain. In this case again the hyperphagia appeared to be the dominant factor.

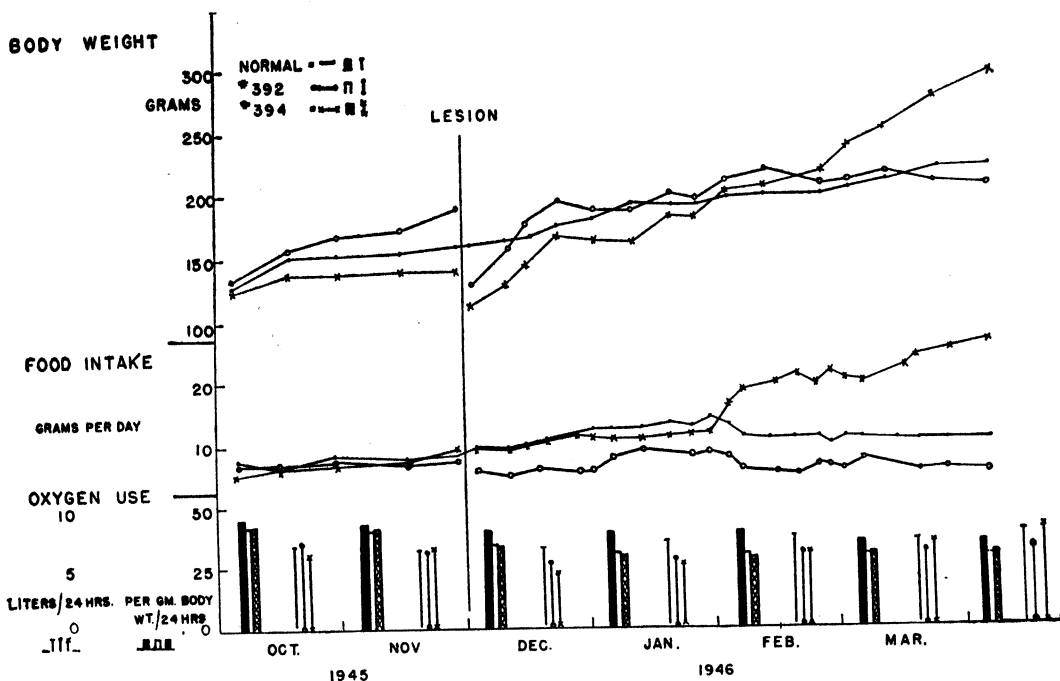


Fig. 2. The oxygen use, food consumption and weight changes of one normal rat, a litter mate with lesions which failed to produce obesity and a second litter mate which was permitted to become obese after a period of food limitation severe enough to prevent it from outgaining the control. Oxygen consumption is shown by the upright lines and columns.

5. *Metabolic compensation in obese rats.* This evidence of a reduced basal rate of energy utilization following hypothalamic injury suggests a slight hypoactivity of the thyroid gland. The heavier weights and the histological appearance of the thyroids from the obese animals tended to support the hypothesis. In testing this assumption that the lesion might have impaired the mechanism controlling the function of the gland 12 obese rats and 5 normal control animals were placed in the cold for 7 to 12 days. The normal rats responded with an increase in basal rate and an augmented activity of the thyroid (12). A few obese animals were totally unable to compensate. Others managed to compensate sufficiently to survive but gave evidence of impairment. Three such animals, despite shivering and increased general motor activity were not able to maintain their temperatures at a normal level. Immediately on removal

from the cold their oxygen consumptions were higher but as soon as shivering stopped the oxygen use fell to the pre-exposure level. The thyroid glands showed no histological evidence of increased activity. The majority of obese animals behaved as did normal rats. Their metabolisms rose and the thyroid glands gave histological evidence of augmented activity. Lesions which impaired or abolished compensation were much larger than those of the compensating rats. They involved not only the nuclei of the midregion of the hypothalamus but also impinged on the anterior, lateral and mammillary regions and so doubtless disturbed the essential central thermoregulatory mechanism.

TABLE 1

Changes in oxygen consumption and body size of pair-fed rats with hypothalamic lesions

DATE	NO. 396 NORMAL CONTROL			NO. 392			NO. 393			NO. 394			PERIODS AVERAGED
	O ₂ /24 hrs.	O ₂ /gm. per 24 hrs.	Body wt.	O ₂ /24 hrs. (% of normal)	O ₂ /gm/ 24 hrs. (% of normal)	Body wt. (% of normal)	O ₂ /24 hrs. (% of normal)	O ₂ /gm/ 24 hrs. (% of normal)	Body wt. (% of normal)	O ₂ /24 hrs. (% of normal)	O ₂ /gm/ 24 hrs. (% of normal)	Body wt. (% of normal)	
1945	<i>l.</i>	<i>cc.</i>	<i>gm.</i>	<i>l.</i>	<i>cc.</i>	<i>gm.</i>	<i>l.</i>	<i>cc.</i>	<i>gm.</i>	<i>l.</i>	<i>cc.</i>	<i>gm.</i>	Oct. Nov.
	6.414	44.44	149	+15.7	+1.0	+18.7	+16.6	-3.9	+17.4	-1.7	-5.6	-6.7	
	6.102	40.32	150	-4.3	-10.7	+8.6	+4.5	+4.1	+2.0	+28.5	-5.9	+42.0	
Length.....	19.7 cm.			19.9 cm.			19.6 cm.			19.6 cm.			Before operation
Girth.....	15.1 cm.			15.6 cm.			14.8 cm.			14.7 cm.			
Lesions made in nos. 392, 393, 394													
1946	6.525	39.11	168	-15.8	-10.4	-6.4	-20.5	-13.3	-7.7	-30.4	-12.7	-19.0	Dec. Jan. Feb. Mar. Apr.
	7.486	39.91	190	-22.7	-26.7	+10.5	-18.1	-17.3	-6.3	-45.9	-27.2	-4.2	
	7.775	37.66	215	-19.8	-21.3	0	-35.6	-48.7	+4.6	-14.5	-30.4	+16.2	
	7.837	35.94	218	-19.7	-17.6	-1.8	-28.8	-32.8	+5.5	-10.4	-18.1	+12.3	
	8.635	38.54	224	-22.6	-28.0	+2.6	-33.2	-40.6	+12.5	-7.2	-24.5	+22.7	
	Normal			Not obese—food intake fell as O ₂ use declined			Slightly obese though pair-fed with 396			Slightly obese though pair-fed with 396 until Feb. Fed ad libi- tum thereafter			
Length.....	22.5 cm.			22.1 cm.			21.3 cm.			21.0 cm.			
Girth.....	17.6 cm.			17.7 cm.			20.6 cm.			21.2 cm.			

These tests demonstrated that some obese animals with lowered basal levels of oxygen utilization can compensate to this degree of exposure to cold. Consequently, if it is assumed that the mechanism affected by the lesions, in producing a lowered basal metabolism, is that which regulates the thyroid gland, it must be recognized that the defect is only a very minor one which does not prevent metabolic compensatory changes.

DISCUSSION. There is no necessary conflict between these results and the conclusions of Newburgh (9), Conn (4), Brucka (3) and others who feel that studies of the metabolism of obese persons have failed to disclose any normal process which accounts for the accumulation of fat. It is within the realm of

possibility that obesity can result from several different causes acting in isolation or in various combinations (10, 2). The basic causes underlying clinically observed obesity must be different in most cases from those causing the experimental obesity dealt with here. Brain lesions certainly are not present in all individuals who have become obese. These experiments clearly show that even in the obesity which results when certain nuclei of the hypothalamus are injured the change in basal rate of energy utilization is not a primary cause nor is it

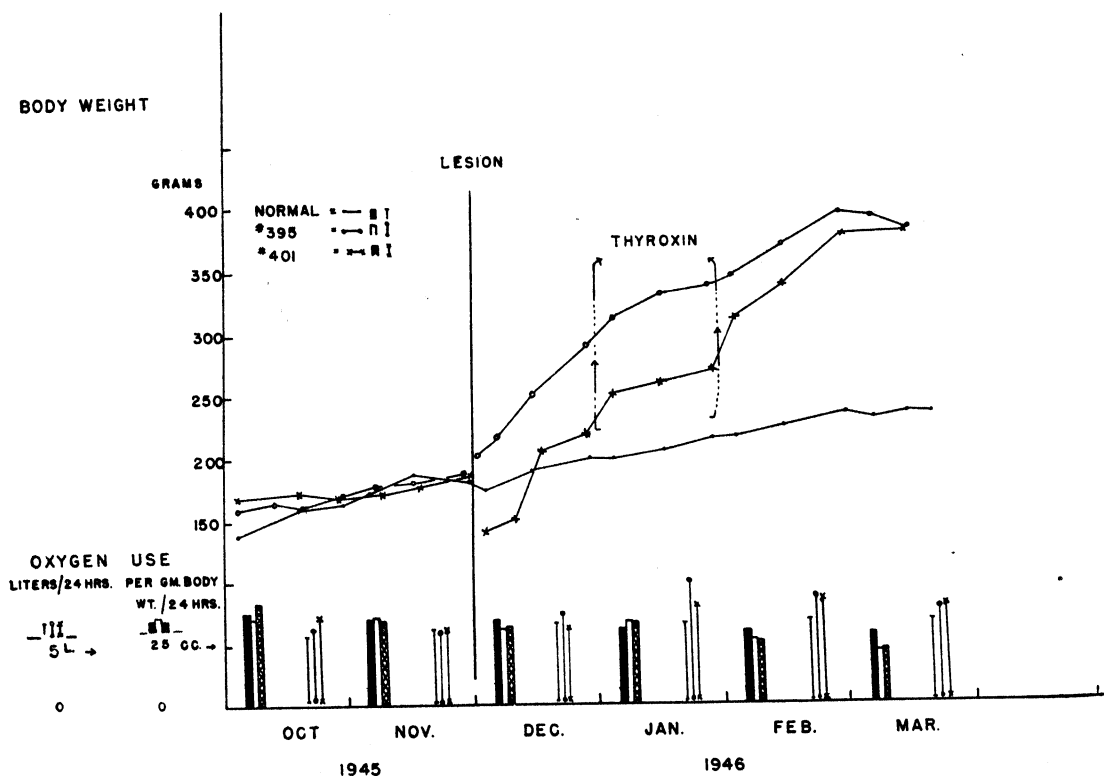


Fig. 3. The average daily oxygen use, the 24 hour oxygen used per gram of body weight and the weight changes of two obese and one normal litter mate. The thyroxine ingested approximated 0.05 mgm. per 200 grams of body weight per day.

essential. A drop in metabolism does occur in some cases and under certain circumstances enables an animal to accumulate more fat than does a normal animal under the same regime. Furthermore, this drop in basal requirement does not contribute to obesity unless it is associated with an abnormality in the control of appetite which prevents the normal adjustment of food intake to energy output. It has been clearly shown again that hyperphagia is the primary causative factor in this type of obesity because raising the metabolic rate retarded but did not prevent weight gain in ad libitum fed rats during the dynamic phase of obesity.

The conclusion that the basal metabolism of certain obese rats was lower than normal is certainly valid in those cases in which the total oxygen consumption was lower than in the controls. The point is less apparent in those cases in which the total oxygen use was greater than in the controls though the oxygen use per unit of weight or surface area was lower. One naturally wonders whether the decrease might not be due to deposition of much metabolically inert matter. The excess weight of obese rats is almost entirely fat but the specialized fat forming tissue is certainly metabolically active; at any rate the blood supply per volume of cytoplasm of fat cells is very rich (5). When compared with animals of similar weights the obese rats always had a lower total oxygen use as well as lower oxygen consumption per unit of tissue. Until they were considerably heavier than the controls all the animals with effective lesions showed a smaller total oxygen consumption after operation. Therefore, it seems unreasonable to assume that their basal rate should suddenly change and become hypernormal as they deposited fat. As long as rats were held within certain weight bounds by limitation of food the total oxygen use remained below normal. Thus there appear to be sufficient reasons to justify the conclusion that the basal metabolism of large obese animals may be somewhat below normal even though their tremendous size necessitates a greater total energy utilization in ordinary activity. It was seen that the increase in size tended to overcome this tendency to gain weight created by the lowered metabolism and the increased energy requirement apparently contributed to the plateauing of body weight. This plateau occurred far short of maximum obesity when food intake was limited.

As regards the means whereby the hypothalamic lesions produce a lowering of the basal metabolic rate, little direct information is available. The thyroid glands were large and showed histological evidence of hypoactivity (2). In many individuals there were lesions present in the adrenal cortex. These observations together with the fact that the gonads of obese male and female rats were of small size might indicate a general lowering of hypophyseal function. For the most part the lesions were caudal to the tuber cinereum and should not have injured the blood supply to the hypophysis or the fibers of the hypothalamico-hypophyseal system which originate in the anterior portions of the hypothalamus. The available evidence indicates that thyroid activity is controlled chiefly by the hypophysis, but certain experimental results suggest a degree of sympathetic control. Whatever the nature of the impairment might be which caused the drop in basal rate after operation it is certain that in some rats the lesions had not destroyed the mechanism which normally causes the thyroid to increase its activity when an animal is exposed to cold.

SUMMARY

1. Comparison of the dry weight of feces with the dry weight of food ingested by normal and obese animals reveals no significantly greater efficiency on the part of the digestive system after hypothalamic injury but a selective change in absorption would have escaped our measurements.

2. Obesity producing hypothalamic lesions do produce, at least in some ani-

mals, a decrease in basal oxygen utilization which, since it is associated with hyperphagia, does contribute to weight gain.

3. This decrease in basal energy requirement may be partially responsible for the ability of some potentially obese animals *a*, to outgain normal rats with which they are pair-fed, and *b*, to gain some weight on a food intake which before operation merely maintained the body weight.

4. The abnormally high metabolic rates produced in some animals by feeding thyroxin retarded but did not abolish weight gain of rats, with effective hypothalamic lesions, which were fed ad libitum.

5. The hypothalamic lesions which evoke obesity do not prevent compensatory metabolic reactions which are elicited by exposure to cold even though the normal basal metabolism may be slightly below normal postoperatively.

REFERENCES

- (1) BROBECK, J. R., J. TEPPERMAN AND C. N. H. LONG. *Yale J. Biol. and Med.* **15**: 831, 1943.
- (2) BROOKS, C. MCC. AND E. F. LAMBERT. *This Journal* **147**: 695, 1946.
- (3) BRUCKA, H. *Am. J. Dis. Child.* **58**: 1001, 1939.
- (4) CONN, J. W. *Physiol. Rev.* **24**: 31, 1944.
- (5) GERSH, I. AND M. A. STILL. *J. Exper. Med.* **81**: 219, 1945.
- (6) HETHERINGTON, A. W. *Endocrinology* **26**: 264, 1940.
- (7) KIBLER, H. H. AND S. BRODY. *J. Nutrition* **24**: 461, 1943.
- (8) NEUENSCHWANDER-LEMMER, N. *Ztschr. f. d. ges. exper. med.* **99**: 394, 1936.
- (9) NEWBURGH, L. H. *Physiol. Rev.* **24**: 18, 1944.
- (10) RONY, H. R. *Obesity and leanness*. Lea and Febiger, Philadelphia, 1940.
- (11) TALBOT, F. B. *Am. J. Dis. Child.* **55**: 455, 1938.
- (12) UOTILA, U. U. *Endocrinology* **25**: 605, 1939.

A STUDY OF THE RESPIRATORY QUOTIENT IN EXPERIMENTAL HYPOTHALAMIC OBESITY^{1, 2}

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The rapid development of obesity following experimental production of hypothalamic lesions in albino rats customarily fed a carbohydrate rich but fat poor diet suggests a very active transformation of carbohydrate to fat. The question immediately arises as to whether or not an exaggerated conversion does occur and whether it is a primary or a secondary factor in the production of excessive adiposity. One of the most direct means thus far devised for measuring the relative quantities of foodstuffs metabolized by the body has been that of determining the respiratory quotient (13). There has been much discussion concerning the validity of the R.Q. as an index of metabolic mixture but the technique is still in use and workers agree generally that though caution should be used in evaluating differences in quotients the ratio is a useful indicator of metabolic events (13, 16). It has been shown that at least during certain phases of the development of obesity the R.Q. following ingestion of carbohydrate rises well above unity (2, 16). Such an R.Q. is characteristic of reactions converting carbohydrate to fat (13).

Arnoldi (1) in a study of a number of obese subjects found that the respiratory quotient occasionally rose above unity. He suggested that obesity might be due to decreased oxidation of glucose and subsequent increase in the synthesis of fat from unused carbohydrate. This concept is at variance with the results of Leites (11) who found that the post-absorptive R.Q. was always within normal limits in obese subjects. It has been stated recently (12) that the respiratory quotients of obese persons both before and after eating are lower than normal. Contrary to these views are the interpretations arising from the equally recent studies by Tepperman, Brobeck and Long (16) in which it was found that unusually high respiratory quotients occur after glucose administration in rats developing hypothalamic obesity. Similar high quotients were observed by Brooks and Bridge (2) in monkeys during the dynamic phase of experimentally produced obesity after ingestion of meals high in carbohydrate content.

It is conceivable that the apparent contradictions in the literature might be due to differences in the types of obesity studied. The metabolic processes involved in the development of the obesity resulting from experimentally produced hypothalamic lesions may be quite different from those involved in the obesity commonly occurring in human beings. It is also possible that these differences in opinion may be related to the fact that different studies have in-

¹ A preliminary report of this work was published in the *Fed. Proc.* 4: 9, 1945.

² The apparatus used in determining the R.Q. was designed and built by Dr. John David Elder, Jr.

volved different stages of obesity. It certainly seems reasonable to consider the suggestion (14, 6) that changes in metabolic processes might be more readily detected during the dynamic phase when imbalance is most apparent than during the static phase when some sort of equilibrium has been re-established. In the experiments to be described here this latter possibility was tested by determining changes in the respiratory quotient in response to ingestion of food throughout the various phases of the obesity which develops as a result of hypothalamic injury.

Tepperman, Brobeck and Long (16) felt that the peculiar R.Q. response of animals with lesions was associated with their unusual eating habits. They were able to develop this response in normal rats by training them to eat their entire day's ration in a short period of time. Even the isolated tissues of such trained rats retained this ability to respond with a high R.Q. when given glucose (9). It was felt that further knowledge of the primary or secondary origin of the response and its rôle in the production of obesity might be obtained by preventing potentially obese animals from eating in their customary manner and by then determining whether or not they developed the observed tendency to give high respiratory quotients following food ingestion.

PROCEDURES. The respiratory quotients of mature rats which had been trained for metabolic studies during several preceding weeks were taken several times during weekly starvation periods of 24 hours. After these intervals of food deprivation the rats were fed measured quantities of dry or moist Purina dog chow or were given saline, glucose, olive oil or amino acid concentrates by stomach tube. In a few individuals glucose was injected intravenously. Three cubic centimeters of a 20 per cent glucose solution were given per 100 grams of rat and calorically equivalent amounts of the other food materials were given on other occasions. Measurements of the R.Q. were made immediately, 30 minutes, 1, 2, 3 and 5 hours after eating or after administration of food materials. When fed, the animals were allowed 15-45 minutes in which to eat before the tests were begun.

The apparatus used in this work was a closed circuit system (6) peculiarly suited to the purpose of measuring the R.Q. at frequent brief intervals following any experimental manipulation. The time required for the use of 50 cc. of oxygen could be readily determined and the carbon dioxide eliminated during the same interval was ascertained by weighing, before and after the test run, the small soda lime tubes through which the air of the system circulated. The quotients thus obtained and used in this paper were uncorrected in that no urine analyses were made.

In those experiments in which the time of eating and the "meal" size were controlled an apparatus was used which has been described elsewhere (3). Hypothalamic lesions were produced by means of a Horsley-Clarke stereotaxic instrument and all procedures of care, feeding, autopsy and tissue study were carried out as in other experiments of this same series (3).

RESULTS. The effects of food ingestion on the respiratory quotient were determined at regular intervals before and after obesity-producing hypothalamic

lesions had been made in twenty adult albino rats. The basal quotients taken after twenty-four hour periods of starvation were not significantly different in the same animal during the normal and the obese state. Basal respiratory quotients of ten obese rats and ten litter mate controls of the same age were likewise practically identical. The similarity of the basal quotients of ten of these animals during normal maturity and the dynamic and static phases of obesity is shown in table 1. In normal animals and throughout the period of normality of the rats which were operated upon the R.Q. rose after ingestion of food but in no instance did the rise exceed a quotient of 0.92. This agrees with

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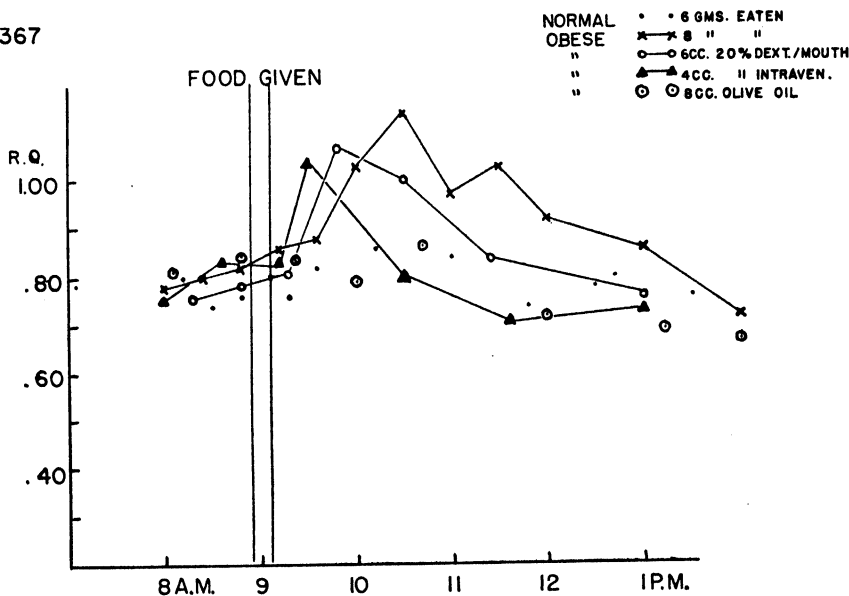


Fig. 1. This figure shows the effects on R.Q. of food before operation and the effects of various food materials given during the dynamic phase of obesity. The times required for attainment of the peak R.Q. after ingestion of Purina dog chow, after giving dextrose by stomach tube and after injecting dextrose intravenously are also shown.

the work of Carpenter and Hartman (8) on monkeys and that of other individuals on various species of animals (13) which has shown that administration of glucose may cause the R.Q. to rise toward but rarely exceed unity.

Immediately following operation the rise in the quotient after glucose or food ingestion was not detectably greater than before but by the third postoperative day some rats gave an R.Q. in excess of 1.0. The highest quotients, however, were obtained after development of obesity was well under way. These abnormally high quotients occurred throughout the dynamic phase of obesity whenever food was given (fig. 1). The highest obtained were 1.20 to 1.22 but averaged response to food or glucose became little if any greater than that which occurred before operation (table 1). Tests done on animals which gained weight slowly and only attained intermediate degrees of obesity were highly unsatisfactory.

At times the R.Q. rose clearly above unity but frequently the responses were only slightly greater than those obtained from control rats. It therefore is apparent that conclusions based on reactions of subjects with minor degrees of obesity or on tests performed during the static phase of obesity will disagree with results obtained during the dynamic phase of the development of maximal obesity. This may offer an explanation of some of the contradictory statements concerning the respiratory quotient in obesity.

While in the dynamic phase, rats which had been starved for twenty-four hours consumed, when allowed to do so, much more food than did similarly starved normal rats during a 45 minute period of exposure to food. This fact raises the question whether the greater rise in R.Q. in the hyperphagic animals is due wholly to the greater amount of food ingested or to some specific alteration in the capacity of the animal with an effective hypothalamic lesion to produce

TABLE 1

The basal respiratory quotients and the peak quotients obtained after eating in ten rats before operation and during the dynamic and static phases of obesity

RAT NO.	NORMAL		DYNAMIC PHASE OF OBESITY		STATIC PHASE OF OBESITY	
	Basal R.Q.	Peak R.Q. after eating	Basal R.Q.	Peak R.Q. after eating	Basal R. Q.	Peak R.Q. after eating
2	0.75	0.81	0.78	1.14	0.76	0.99
21	0.70	0.79	0.74	1.01	0.72	0.89
302	0.79	0.89	0.79	1.09	0.78	0.87
303	0.74	0.86	0.79	1.04	0.75	0.78
332	0.78	0.83	0.79	1.09	0.76	0.88
364	0.75	0.79	0.78	1.19	0.75	0.92
365	0.74	0.88	0.77	1.09	0.77	0.89
367	0.76	0.86	0.82	1.14	0.78	0.91
368	0.70	0.88	0.72	1.08	0.75	0.81
371	0.76	0.82	0.78	1.15	0.80	0.88
Average	0.75	0.84	0.78	1.10	0.76	0.88

fat from carbohydrates or possibly from proteins. Obviously, if in these experiments a rise in R.Q. signifies a conversion of carbohydrate to fat, this transformation must proceed at a rate which suffices to outstrip that of all processes which tend to lower the quotient. Consequently, there must be a minimal quantity of ingested food necessary to cause such a rise in the R.Q. The ingestion of an amount of food greater than this threshold amount should, therefore, raise the R.Q. still further. A series of tests revealed that the rise above unity was not detectable unless three grams or more, dry weight, of food (Purina dog chow) were ingested. Larger quantities caused a slightly higher rise but chiefly a prolongation of the period of high R.Q. The rise was detectable 60 minutes after eating a four to six gram meal and the peak was attained within 2 hours. Within four hours the R.Q. generally fell to or below the pre-feeding level. In figure 2 is shown one series of results typical of those on which these generalizations are based.

No attempt was made to determine the minimum quantity of glucose capable of producing a detectable rise of the R.Q. but when glucose was given by stomach tube to rats in the dynamic phase of obesity the elevation of the R.Q. reached a maximum within 45 minutes. When glucose was injected intravenously the R.Q. rose above unity within 30 minutes after injection. It is apparent that the sooner the carbohydrate reaches the sites of fat formation the earlier is the change in R.Q. (fig. 1).

One indication that the high R.Q. represents conversion of carbohydrate to fat and is not the result of some artefact of the experimental procedure was furnished by the observation that saline or eight to ten cubic centimeters of olive oil when given by stomach tube produced no rise in the quotient (fig. 1). Neither

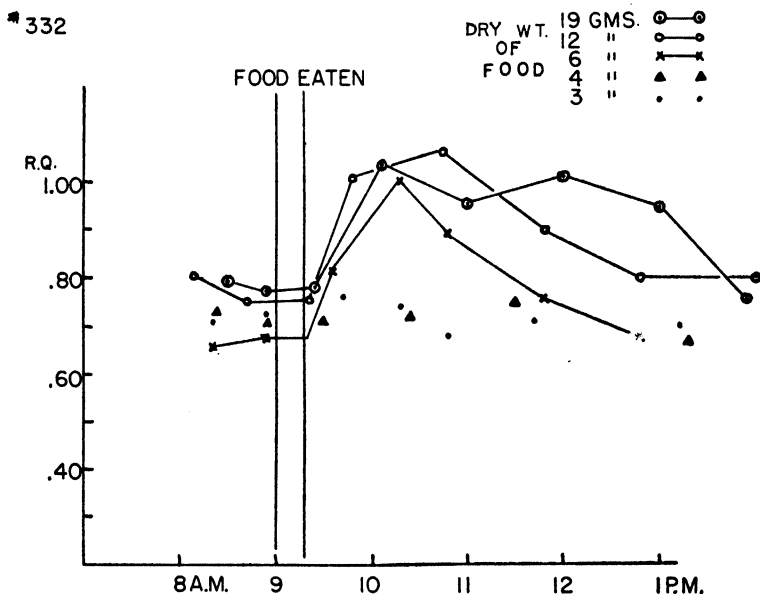


Fig. 2. The effects of varying the amount of food ingested during a meal on the height and duration of the postprandial rise in R.Q. of a rat during the dynamic phase of obesity.

substance had any clearly detectable effect and the R.Q. remained relatively unchanged as expected (13). It was thought, however, that protein ingestion might produce a rise in the R.Q. if enough could be given to provide a source of carbohydrate sufficient to meet the basic requirements for development of a detectable conversion to fat. Injections by stomach tube of 10 to 15 cc. of a 15 per cent mixed amino acid solution (Parenamine—Frederick Sterns and Co.) had little effect but this amount of protein was insufficient to furnish the minimum required carbohydrate as judged from the amount of carbohydrate in the minimum effective meal. When the solution was concentrated and 10 to 15 cc. of this 30 per cent amino acid solution were given the animals were rendered lethargic and a few died. Because of the apparent toxicity of this concentrated preparation this means of supplying potentially convertible material was

abandoned. It was not possible to show that protein ingestion raised the respiratory quotient as did carbohydrate.

In investigating the effect of eating habits on the R.Q. values a series of six rats, with effective hypothalamic lesions, were pair-fed with normal rats and all were given their total daily ration subdivided in small quantities which never exceeded two grams. These portions were delivered to the rats at regularly spaced intervals. Since this meal size was equal to the previously determined meal size of normal rats (5) the amount of food given could hardly create a plethora of carbohydrate capable of producing an abnormal storage. Furthermore, the small meals were sufficiently close together to prevent a compensation typical of starvation. Nevertheless, these rats, when given a test meal (Purina dog chow) or glucose, showed a rise in the respiratory quotient to a level above unity. Thus the phenomenon developed although periodic engorgement with food had never occurred. None of these pair-fed and spaced-fed animals gave as high quotients after eating as did several of the rats which were fed *ad libitum* but the numbers of animals tested and the degrees of variation commonly encountered were so great that no significance can be attached to this lower intensity of response without a great amount of additional information.

It has been stated that a second dynamic phase can be produced by starving fully obese animals until their weight is greatly reduced and then giving them unlimited food (14). Such experiments were performed (3) and it was found that following starvation food consumption rose and weight was regained at a rapid rate. In no case, however, did the animals show as great a rise in R.Q. after food and glucose ingestion as they had during the original postoperative dynamic period of weight gain. Quotients of 1.01–1.03 were obtained and these are higher than those obtained during the normal preoperative period or the prestarvation static phase of the obesity. None of the reactions of the poststarvation "dynamic phase" was equal to those previously observed following operation.

DISCUSSION. If the exaggerated rise in R.Q. which occurs during the dynamic phase of experimentally produced obesity following food ingestion does indicate an unusually active conversion of carbohydrate to fat it is still difficult to estimate its significance. Evidence is certainly insufficient to warrant consideration of this disturbance in intermediate metabolism to be a major cause of obesity. Hyperphagia should be considered the major cause until it is known just what metabolic or neural abnormalities are responsible for the abnormal appetite. It might be that the change in R.Q. is a consequence of the same basic disturbance which causes the appetite to lose its normal relationship to energy requirements of the animal.

If these experiments, in which engorgement and a subsequent long period of food deprivation were prevented, can be considered adequate, they bring into question the contention that the exaggerated R.Q. response is secondary to the eating habit induced by the change in appetite. Even if the R.Q. rise were secondary to the peculiar method of eating one still does not know exactly how

the cells were induced to acquire the property of reacting as they do. Certain possibilities are worthy of consideration even though they are merely hypotheses.

Once large numbers of fat forming cells are differentiated or once other cells develop the need to manufacture fat the high R.Q. response would tend to occur whenever a supply of carbohydrate becomes available. It has been shown that new formation of fat is stimulated by fat deficient diets (7). If the need for fat could be created by a partial blocking of the normal breakdown and use of stored fat then there might result an exaggeration of new fat formation. Furthermore, if those processes which normally give a low R.Q., such as the burning of fat, should be impaired then a normal rate of conversion of carbohydrate to fat might appear to be an exaggeration.

There can be no question as to the fact that obese rats can burn some fat because they lose weight and fat when starved. There is reason to believe, however, that fat use may be impaired and several individuals have suggested this as one possible cause of obesity (14, 10, 15). Salcedo and Stetten (15) fed labeled fatty acids and studied fat deposition and the rate of burning of depot fats in normal and obese mice. New formation of fat was not impaired in the obese animals but they did burn rather less quantities of depot fat. These investigators felt that the obesity might be attributed to the retarded catabolism of stored fatty acids. Such a condition as this might explain the considerable rise of the R.Q. on administration of food during the dynamic phase of obesity. As fat is deposited the differentiated fat forming or storing cells could conceivably lose their ability to form new fat as they become engorged; as fat stores become enormous even the possibly impaired mechanisms for breaking down stored fat might be sufficient to supply all the fat that the cells can use and thus gradually impair the urge for new fat formation. Such a condition would prevail during the static phase of obesity and at that time food ingestion does not produce the abnormally high rise in R.Q. Even if these suggestions should prove to be acceptable at least in part there would still remain the problem as to how hypothalamic lesions impair fat metabolism and why they should affect appetite.

SUMMARY

The basal respiratory quotients of rats which responded to injury to the ventromedian areas of the hypothalamus by becoming obese were not significantly higher than the quotients of normal controls.

During the dynamic phase of obesity the ingestion of food or glucose solutions by the obese rats raised the R.Q. above unity. During the static phase the R.Q. responses to food and glucose ingestion were not essentially different from those of normal animals. Nevertheless, during a second dynamic phase produced by temporary starvation a typically abnormal rise in R.Q. could again be evoked by giving food. This response, however, was never as great as that obtained during the initial dynamic period.

Two to three days were required for development of the exaggerated R.Q. response. The minimal amount of food required for its elicitation was found to

be 3 grams of dry Purina dog chow. Larger amounts of food augmented the height of the R.Q. response only slightly but prolonged its duration markedly.

Ingestion of saline, olive oil and protein did not raise the R.Q. significantly.

The rise in the quotient appeared most promptly when glucose was injected intravenously. Glucose given by stomach tube required a slightly longer time to evoke the response. Food ingestion gave the most delayed but the longest lasting elevation of the R.Q.

Spaced feeding of pair-fed animals with effective hypothalamic lesions did not prevent development of the abnormal rise in R.Q. in response to feeding. In these experiments the phenomenon appeared even though periodic engorgement with food followed by long periods of fasting had never occurred.

REFERENCES

- (1) ARNOLDI, W. *Ztschr. f. klin. med.* **94**: 268, 1922.
- (2) BROOKS, C. McC. AND E. M. BRIDGE. *Endocrinology* **35**: 208, 1944.
- (3) BROOKS, C. McC. AND E. F. LAMBERT. *This Journal* **147**: 695, 1946.
- (4) BROOKS, C. McC., E. F. LAMBERT AND P. BARD. *Fed. Proc.* **1**: 11, 1942.
- (5) BROOKS, C. McC., R. A. LOCKWOOD AND M. L. WIGGINS. *Fed. Proc.* **4**: 9, 1945.
- (6) BROOKS, C. McC., D. N. MARINE AND E. F. LAMBERT. *This Journal* **147**: 717, 1946.
- (7) BURR, G. D. AND A. J. BEBER. *Proc. Soc. Exper. Biol. and Med.* **31**: 911, 1933.
- (8) CARPENTER, T. M. AND C. G. HARTMAN. *This Journal* **141**: 249, 1944.
- (9) DICKERSON, V. C., J. TEPPERMAN AND C. N. H. LONG. *Yale J. Biol. and Med.* **15**: 875, 1943.
- (10) HETHERINGTON, A. W. AND S. W. RANSON. *This Journal* **136**: 609, 1942.
- (11) LEITES, S. M. *Acta Med. Scand.* **89**: 199, 1936.
- (12) NEWBURGH, L. H. *Physiol. Rev.* **24**: 18, 1944.
- (13) RICHARDSON, H. B. *Physiol. Rev.* **9**: 61, 1929.
- (14) RONY, H. R. *Obesity and leanness*. Lea and Febiger, Philadelphia, 1940.
- (15) SALCEDO, J. AND DEW. STETTEN. *J. Biol. Chem.* **151**: 413, 1943.
- (16) TEPPERMAN, J., J. R. BROBECK AND C. N. H. LONG. *Yale J. Biol. and Med.* **15**: 855, 1943.

A STUDY OF THE EFFECT OF HYPOTHALAMIC LESIONS ON THE EATING HABITS OF THE ALBINO RAT¹

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It has become apparent that the major defect created by hypothalamic lesions which produce obesity is that of breaking down the normal relationship between food intake and energy output (1, 3). These lesions cause other deviations from normality which contribute to the deposition of excess fat but they are effective only because the appetite is not appropriately modified to compensate for a reduction in activity (2) and in basal metabolism (4). It was felt that more might be learned concerning the cause of the abnormal appetite and the factors responsible for obesity if the changes in eating habits induced by the hypothalamic lesions and various other procedures were investigated. Consequently, a study was made of the eating habits of rats before and after operation. Attempts also were made to determine the effects of surgical reduction of the size of the stomach and of section of the vagi on the hyperphagia.

PROCEDURES. Figure 1 shows a device which was built to record the time of eating and the amounts of food eaten at each meal. It can be seen from this diagram that the distended spring was able to contract proportionately as the animal ate food from the cup. A writing point attached to the end of the lever arm recorded this weight decrement on a slowly rotating drum. The sensitivity of the recording system was such that a movement of 1 mm. by the pointer equalled 1 gram of food eaten.

The 24-hour food consumption was determined and the amount eaten within the first hour after fresh food had been given was also measured. The newly prepared food, which consisted of Purina dog chow mixed with an equal weight of water, was placed in the cages each day between 9 and 10 a.m. Enough of this mash was given to make sure that the rats were never without food during the 24 hours. Thus they were fed *ad libitum*.

All surgical procedures were carried out on animals which were anesthetized with ether or Evipal. In those cases in which the vagi were cut ether was used and both nerves were severed low in the chest just above the diaphragm. To prevent quick regeneration three or four millimeters of the nerve trunks were removed. In some instances both vagi were cut during one operation but the rats survived more frequently when the nerves were transected singly with a period of two to three weeks intervening between operations. It was thought that traction exerted upon the partially denervated oesophagus by action of the diaphragm might interfere with passage of food into the stomach. In order to eliminate this possibility the left phrenic nerve was cut in four of the rats at the

¹ A preliminary account of this work was published in the Fed. Proc. 4: 9, 1945.

time of vagus section. All vagotomies were performed after obesity-producing hypothalamic lesions had been made and while the rats were in the dynamic phase of obesity. The method of producing the hypothalamic lesions has been described elsewhere (3).

Reduction of the volume of the stomach was accomplished either by removing a large section from the middle of the organ and approximating the cardiac and pyloric remnants or by making a tube along the lesser curvature as done by Tsang (6). In this way the stomach was reduced in volume from one-fifth to one-tenth of its original size. Gastrectomies were performed before hypothalamic lesions were made in some cases but in others not until obesity had begun to develop. After gastrectomy, rats were kept on a fluid diet for a few days then permitted to eat mash as before operation. A few rats which refused to eat mash were given hard dog biscuits which they did eat. After operation all the animals seemed to prefer the biscuits to the soft mash (6).

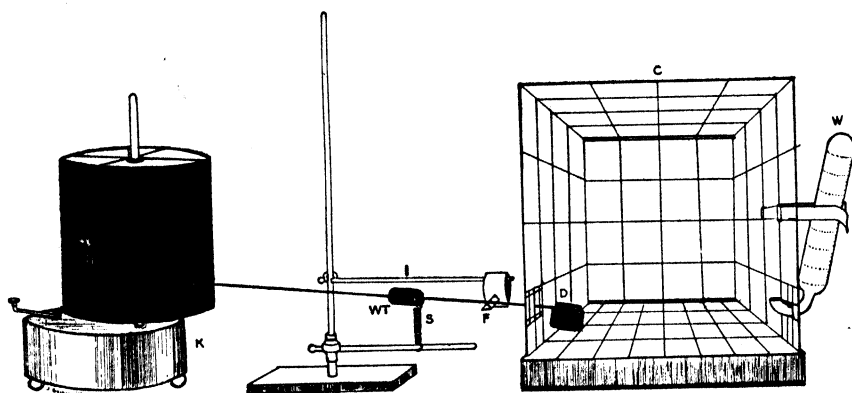


Fig. 1. Apparatus for recording time of eating and meal size. *C*, cage; *D*, food dish; *F*, fulcrum; *K*, kymograph; *S*, spring; *Wt*, weight to counterbalance food cup.

RESULTS. All rats studied, when normal, ate approximately 70 per cent of their daily food intake at night (6 p.m. to 6 a.m.). During the day they ate, on the average, once every 3 to 4 hours but at night the frequency was once every 1 to 2 hours. The maximal meal size rarely exceeded 4 grams of mash and the average was 1.5 to 2.0 grams. Day and night meals did not vary greatly in size but the largest meal of the 24-hour period was generally eaten at night. When, each morning, fresh food was given and the animals were disturbed by cage cleaning, etc. they customarily ate a little food but the meal was never the largest meal of the day. Figure 2 and table 1 present examples of the evidence used in formulating these conclusions.

Hypothalamic lesions of the type which produce obesity reversed the period of maximal food consumption. During the dynamic phase of obesity animals habitually ate an enormous meal (14 to 20 grams) immediately after the food container was filled with fresh food. This resulted in a daytime consumption of approximately 65 per cent of the total daily food intake. Throughout the

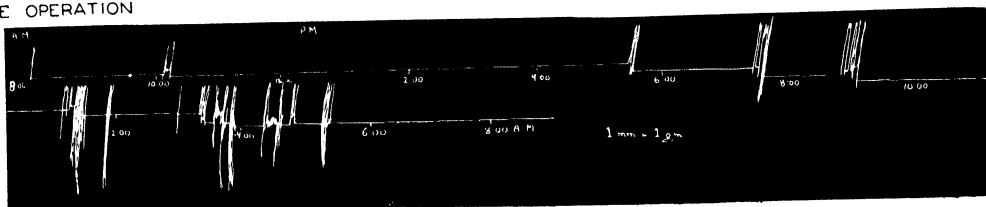
remaining hours after this large initial meal the rats ate at rather uniform intervals of once every 2 hours. The average meal size was 4 to 7 grams.

The postoperative increment in appetite appeared too rapidly to have been the result of some peculiar food need or deficiency resulting from any endocrine or digestive malfunctioning. Immediately after the operation and before the animals were sufficiently recovered from the anesthetic (Evipal) to stand and walk normally they began to eat. As reported by Brobeck, Tepperman and Long (1) this tendency of the rats to gorge themselves with food frequently resulted in death and we consequently made a practice of limiting the food during the first postoperative day.

Associated with this intense hyperphagia was an overactivity (5) and a hypersensitivity or emotional hyperresponsiveness which practically precluded the

TIME AND AMOUNT OF FOOD CONSUMPTION

BEFORE OPERATION



DYNAMIC PHASE

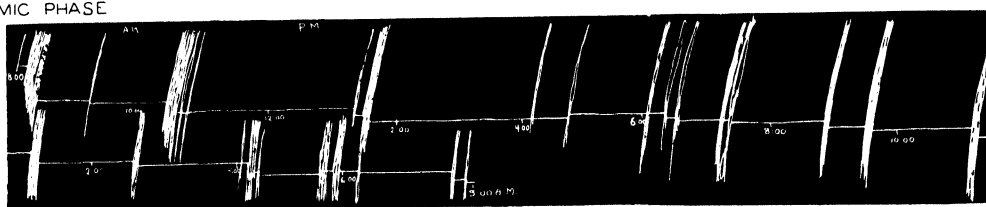


Fig. 2. Records of eating taken before operation and after operation during the dynamic phase of obesity.

handling of the animals. It was thought that this emotional imbalance might be responsible for the hyperphagia. Throughout this immediate postoperative period especially, but also during the remainder of the lives of these animals any trifling disturbance caused them to bite, struggle and eat. Whenever one of these rats was angered by handling and then returned to its cage it immediately attacked its food cup and usually began to eat. This emotional hyperresponsiveness tended to disappear gradually as the animals became obese but a majority, if not all, never regained a fully normal emotional stability.

It was felt that the reversal of the eating habit might have been due to the fact that the rats were fed fresh food and disturbed in the morning. The first morning meals were so large that they tended to be responsible for the greater proportion of daytime eating. This point was tested by giving the fresh food at different times of the day. As shown in table 2 the animals did tend to eat the largest

meal just after feeding no matter what the time of day or night. This occurred but to a lesser extent when the food cups were refilled with old mash instead of freshly prepared food. Activity measurements (2) strengthened the conclusion that these rats with lesions tended to fill their stomachs with food, then be

TABLE 1

Time of eating, meal size and the total food intake of five animals when normal and during the dynamic and static phases of obesity

RAT	PHASE	CHANGE IN BODY WEIGHT	AVE. DAILY FOOD IN- TAKE	AVE. NO. OF MEALS	AVE. EATEN PER MEAL	PER CENT OF TOTAL FOOD CON- SUMED	FOOD EATEN IN 2-HR. INTERVALS (FRESH FOOD GIVEN AT 10 a.m.)																PERIOD AVER- AGED
							Day	Night	a.m. 10-12	p.m.						a.m.							
										12-2	2-4	4-6	6-8	8-10	10-12	12-2	2-4	4-6	6-8	8-10			
No. 19	Normal Dynamic Static	gms.	gms.		gms.																	days	
		278-283	35.0	11	3.2	28	72	1.7	.95	1.0	1.8	2.4	5.4	3.5	5.0	5.1	5.0	2.9	2.3	14			
		281-382	69.8	9	7.8	61	39	10.9	6.1	7.2	5.7	5.2	4.1	4.5	5.1	5.5	4.4	4.6	4.8	21			
No. 281	Normal Dynamic Static	382-390	49.7	10	4.9	49	51	5.8	4.4	4.5	4.7	4.0	3.3	5.3	4.0	4.6	3.9	2.1	3.1	14			
		220-228	24.5	8	3.1	33	67	1.3	1.1	1.3	1.2	3.3	2.6	2.6	3.5	2.2	2.1	1.5	1.8	7			
		390-430	42.2	10	4.2	54	46	7.7	2.5	1.7	4.1	2.1	3.6	2.8	2.7	4.5	3.3	3.8	3.4	7			
No. 284	Normal Dynamic Static	430-426	29.4	8	3.5	43	57	3.4	1.2	1.0	2.6	2.4	4.0	2.9	3.0	2.2	2.2	1.7	1.9	14			
		250-255	44.5	12	3.7	34	66	2.3	3.6	2.8	1.7	5.3	4.6	3.6	6.5	4.2	5.1	3.0	2.8	7			
		350-412	62.0	12	5.2	58	42	10.9	5.1	5.5	8.0	3.8	5.8	4.0	4.2	4.1	4.0	3.8	2.8	14			
No. 295	Normal Dynamic Static	412-418	54.4	12	4.5	45	55	6.2	4.4	6.6	7.1	5.6	3.0	4.3	3.9	4.0	3.9	2.9	2.5	7			
		350-370	36.6	11	3.3	27	73	1.7	.95	1.0	1.8	2.4	5.4	3.5	5.0	5.1	5.0	2.9	2.3	21			
		332-438	66.9	6	11.1	61	39	20.3	4.1	5.2	3.6	5.1	4.5	2.7	4.5	3.7	4.6	3.9	4.2	28			
No. 297	Normal Dynamic Static	438-428	34.7	8	4.3	38	62	1.8	2.1	1.2	2.6	2.1	3.3	4.0	3.5	3.0	5.6	4.0	1.5	7			
		293-300	42.6	9	4.7	45	55	0.6	5.0	4.5	6.4	1.5	4.1	5.1	6.6	3.9	2.0	1.2	1.7	7			
		300-335	81.9	9	9.1	54	46	13.5	6.3	2.0	8.8	2.7	4.5	13.5	4.5	9.0	2.8	6.3	8.0	7			
No. 297	Normal Dynamic Static	350-352	39.0	10	3.9	39	61	1.5	5.5	4.0	2.0	8.5	3.5	4.5	3.0	2.5	1.5	1.0	1.5	7			

TABLE 2

The relationship of the period of greatest food consumption to the time at which fresh food was given

CONDITION	DATE	AVE. DAILY FOOD INTAKE	FOOD EATEN IN 2-HR. INTERVALS																TIME AT WHICH FOOD WAS GIVEN	
			a.m. 10-12	p.m.								a.m.								
				12-2	2-4	4-6	6-8	8-10	10-12	12-2	2-4	4-6	6-8	8-10						
Becoming obese.	7/23/42	53.8	14.0		8.7		8.5	4.0	4.0		5.3	4.0	5.3					10 a.m.		
Body wt. 382 grams	7/26/42	64.5	3.0	1.0	22.6	4.1	9.5		3.3		11.0		8.0	2.0				2 p.m.		
	7/30/42	57.9	4.0		2.0	1.3	19.3	6.8		10.0		12.5		2.0				6 p.m.		
	8/10/42	50.6	3.0		6.0	1.0		13.3	2.0	6.7	4.0	9.3		5.3				8 p.m.		
	8/12/42	63.3	7.5	1.0	8.0		2.2		5.2	3.4	17.0	8.0	10.0	1.0				2 a.m.		

inactive and sleep until they could eat again. Their greater liking of freshly prepared food and the disturbances associated with daily feeding and cage cleaning were thought to be responsible for the largeness of the initial post-feeding meals and the emphasis on food consumption during the day rather than the night.

During the first day after operation the average meal size and therefore the food intake were not quite as great as on subsequent days. The total intake and average meal size increased steadily from day to day as though the capacity of the stomach and intestines was increasing. A peak was reached some days before the end of the dynamic phase and a fall then ensued which continued until a level of intake slightly above normal was reached (3). The pattern of eating also tended to revert to the normal as the static phase of obesity was attained. At night 56 per cent of the food was eaten despite the fact that fresh food was still given in the morning. Meal size averaged 3 to 4 grams and the average frequency of eating was once every 2.5 to 3 hours during the day and once every 2 hours at night. Emotional stability tended to be re-established to a considerable degree.

When the intensity of appetite was judged by measuring the average percentage of the total daily food intake which was eaten by 20 animals during the first 2 hours after feeding in the morning, the following results were obtained: during the preoperative period, 11.5 per cent of normality; during the dynamic phase of obesity, 31.3 per cent; 19.3 per cent during the static phase and 13.4 per cent after signs of senescence were detectable in the obese animals. This crude method of estimating appetite thus shows the sudden increment following operation and the gradual waning characteristic of the static phase of obesity and senescence.

It was thought that if the exaggerated size of the stomach were responsible in part for the continuation of hyperphagia, surgical reduction of the stomach might affect weight gain and appetite. The stomachs of 2 normal rats and 6 animals which were in the dynamic phase of obesity were reduced approximately from one-fifth to one-tenth of their previous capacity. All these animals showed a reduced food intake and a weight loss which lasted from one week to ten days. They all eventually compensated for their smaller gastric capacity by eating smaller meals more frequently and the 24-hour food consumption soon attained the preoperative level. Before gastrectomy the average meal size of the 6 rats which were becoming obese was 4.2 grams. During the first days of spontaneous eating after gastrectomy the average meal size was 0.5 to 0.7 gram; during the second and third week after operation the average rose to 1.0–1.5 grams. Eventually as the gastric remnant hypertrophied the rats were able to consume meals of 4 to 5 grams but the average meal size remained 1.8 to 2.5 grams. At autopsy it was found, as shown by Tsang (6), that although the stomach remnants had not enlarged greatly the upper part of the duodenum had become considerably distended as though it were being used to supplement the stomach remnant. These six animals eventually became typically obese despite the reduction of gastric capacity.

The subtotal gastrectomies must have interfered with the innervation of the stomach but they did not abolish hyperphagia and obesity. The question of the rôle of gastric innervation in the hyperphagia was followed further in other potentially obese rats by transecting both vagi and observing the effect on food consumption. Double vagotomies were performed in ten rats which had effec-

meal just after feeding no matter what the time of day or night. This occurred but to a lesser extent when the food cups were refilled with old mash instead of freshly prepared food. Activity measurements (2) strengthened the conclusion that these rats with lesions tended to fill their stomachs with food, then be

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								a.m. 10 12		p.m.								a.m.									
										Day	Night	12 2	2 4	4 6	6 8	8 10	10 12	12 2	2 4	4 6	6 8	8 10					
No. 19	Normal Dynamic Static	<i>gms.</i> 278-283	<i>gms.</i> 35.0	11	<i>gms.</i> 3.2	28	72	1.7	.95	1.0	1.8	2.4	5.4	3.5	5.0	5.1	5.0	2.9	2.3	<i>days</i> 14							
		281-382	60.8		9	7.8	61	39	10.9	6.1	7.2	5.7	5.2	4.1	4.5	5.1	5.5	4.4	4.6		4.8						
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		430-426	29.4	8	3.5	43	57	3.4	1.2	1.9	2.6	2.4	4.0	2.9	3.0	2.2	2.2	1.7	1.9	14							
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		300-335	81.9	9	9.1	54	46	13.5	6.3	2.0	8.8	2.7	4.5	13.5	4.5	9.0	2.8	6.3	8.0	7							
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				12-2	2-4	4-6	6-8	8-10	10-12	12-2	2-4	4-6	6-8	8-10						
Becoming obese.	7/23/42	53.8	14.0		8.7		8.5	4.0	4.0		5.3	4.0	5.3				10 a.m.			
Body wt. 382	7/26/42	64.5	3.0	1.0	22.6	4.1	9.5		3.3		11.0		8.0	2.0			2 p.m.			
grams	7/30/42	57.9	4.0		2.0	1.3	19.3	6.8		10.0		12.5		2.0			6 p.m.			
	8/10/42	50.6	3.0		6.0	1.0		13.3	2.0	6.7	4.0	9.3		5.3			8 p.m.			
	8/12/42	63.3	7.5	1.0	8.0		2.2		5.2	3.4	17.0	8.0	10.0	1.0			2 a.m.			

inactive and sleep until they could eat again. Their greater liking of freshly prepared food and the disturbances associated with daily feeding and cage cleaning were thought to be responsible for the largeness of the initial post-feeding meals and the emphasis on food consumption during the day rather than the night.

During the first day after operation the average meal size and therefore the food intake were not quite as great as on subsequent days. The total intake and average meal size increased steadily from day to day as though the capacity of the stomach and intestines was increasing. A peak was reached some days before the end of the dynamic phase and a fall then ensued which continued until a level of intake slightly above normal was reached (3). The pattern of eating also tended to revert to the normal as the static phase of obesity was attained. At night 56 per cent of the food was eaten despite the fact that fresh food was still given in the morning. Meal size averaged 3 to 4 grams and the average frequency of eating was once every 2.5 to 3 hours during the day and once every 2 hours at night. Emotional stability tended to be re-established to a considerable degree.

When the intensity of appetite was judged by measuring the average percentage of the total daily food intake which was eaten by 20 animals during the first 2 hours after feeding in the morning, the following results were obtained: during the preoperative period, 11.5 per cent of normality; during the dynamic phase of obesity, 31.3 per cent; 19.3 per cent during the static phase and 13.4 per cent after signs of senescence were detectable in the obese animals. This crude method of estimating appetite thus shows the sudden increment following operation and the gradual waning characteristic of the static phase of obesity and senescence.

It was thought that if the exaggerated size of the stomach were responsible in part for the continuation of hyperphagia, surgical reduction of the stomach might affect weight gain and appetite. The stomachs of 2 normal rats and 6 animals which were in the dynamic phase of obesity were reduced approximately from one-fifth to one-tenth of their previous capacity. All these animals showed a reduced food intake and a weight loss which lasted from one week to ten days. They all eventually compensated for their smaller gastric capacity by eating smaller meals more frequently and the 24-hour food consumption soon attained the preoperative level. Before gastrectomy the average meal size of the 6 rats which were becoming obese was 4.2 grams. During the first days of spontaneous eating after gastrectomy the average meal size was 0.5 to 0.7 gram; during the second and third week after operation the average rose to 1.0–1.5 grams. Eventually as the gastric remnant hypertrophied the rats were able to consume meals of 4 to 5 grams but the average meal size remained 1.8 to 2.5 grams. At autopsy it was found, as shown by Tsang (6), that although the stomach remnants had not enlarged greatly the upper part of the duodenum had become considerably distended as though it were being used to supplement the stomach remnant. These six animals eventually became typically obese despite the reduction of gastric capacity.

The subtotal gastrectomies must have interfered with the innervation of the stomach but they did not abolish hyperphagia and obesity. The question of the rôle of gastric innervation in the hyperphagia was followed further in other potentially obese rats by transecting both vagi and observing the effect on food consumption. Double vagotomies were performed in ten rats which had effec-

tive hypothalamic lesions. Four of these animals died within two to three weeks after section of both nerves had been completed. All of the rats were ill for many days and lost much weight. They would endeavor to eat but the food would be partially regurgitated. In those animals which died the oesophagus was enormously distended with fluid and food and the stomach was flaccid and enlarged. The rats which survived gradually began to eat more and retain more food and weight loss ceased. Reduction of the activity of the diaphragm apparently had little effect on the emptying of the esophagus.

The most prominent change observed in eating habit following vagotomy was an irregularity in performance. The time of eating and the meal size fluctuated much more than in normal and obese animals with intact vagi. The only rats to become fat were four in which remnants of vagal connections with the stomach were found at autopsy. It is possible that these remnants or regenerated neurons had aided the animals to recover from vagotomy even though they did not constitute a complete restoration of the innervation. In the two remaining rats no vagal remnants were found and electrical stimulation of the thoracic trunks caused no visible contractions of the gastric musculature. These rats did not become obese although the nature of the lesions was such that mild degrees of obesity would have been predicted. On the basis of this evidence it cannot be said that the vagal innervation is essential for the development of the abnormal appetite. Vagotomy involves the severance of motor as well as sensory fibers. The motor effects were so profound that they may have prevented the obesity and hyperphagia.

DISCUSSION. The intensity of the appetite which develops following obesity-producing hypothalamic injury is such that animals tend to lose their normal cycle of eating and tend to consume food at a rather steady rate throughout the day. It is conceivable that the observed inclination on their part to attack and consume food because of a sensory or emotional abnormality created by the lesion might explain the hyperphagia. Even a disturbance of temporary duration might act as a sufficient stimulus by causing animals to ingest so much food during the first few days that they developed distended stomachs and abnormal eating habits. It is conceivable that after a period of distention a greater volume of food is required to prevent hunger contractions from appearing in the stomach. This question was not followed up but it can be said that in experiments in which food intake was limited and engorgement was prevented by spaced feeding only those animals became obese which retained symptoms of emotional instability.

A few rats in addition to developing an obesity became somnolent and inactive after operation and could be easily handled. This brings into question any hypothesis which suggests that the emotional instability may have caused hyperphagia. No quantitative tests of sensitivity, emotional stability or responsiveness were made and until that has been done little can be said concerning the presence or absence of this emotional instability in somnolent animals.

Appetite apparently was not impaired by reduction of the size of the stomach. Such operations must have interfered with the nerve supply but this apparently had no effect. Section of the vagi caused both normal and obese rats to lose

weight but this could well have been due to the motor paralysis which prevented normal handling of food by the gastrointestinal system. These experiments do not settle the question as to whether overactivity of the stomach or lack of a sense of distention and satiation mediated by vagal afferents may contribute to the production of obesity but they do render these hypotheses rather untenable.

SUMMARY

The time of eating and the amount of food habitually consumed during one eating period were determined in rats before and after hypothalamic lesions had been made which resulted in obesity.

Rats which were becoming obese showed an increase in average meal size. Unlike normal rats these animals ate more food during the day than during the night.

The abnormality in appetite which resulted in hyperphagia appeared immediately after the hypothalamic lesions were made. There was a gradual waning of the intensity of this hyperphagia as the static phase of obesity developed.

Reduction in the size of the stomach did not prevent development of obesity. The rats ate smaller meals but they ate more frequently.

Bilateral vagotomy resulted in a greater variation in time of eating and meal size than observed in normal and obese animals with intact vagi. Studies of the effect of vagotomy on appetite and the development of obesity gave inconclusive results.

REFERENCES

- (1) BROBECK, J. R., J. TEPPERMAN AND C. N. H. LONG. *Yale J. Biol. and Med.* **15**: 831, 1943.
- (2) BROOKS, C. McC. *This Journal* **147**: 708, 1946.
- (3) BROOKS, C. McC. AND E. F. LAMBERT. *This Journal* **147**: 695, 1946.
- (4) BROOKS, C. McC., D. N. MARINE AND E. F. LAMBERT. *This Journal* **147**: 717, 1946.
- (5) HETHERINGTON, A. W. AND S. W. RANSON. *This Journal* **136**: 609, 1942.
- (6) TSANG, Y. *J. Comp. Psychol.* **26**: 1, 1938.

THE EFFECTS OF INSULIN ON THE INCREASE IN LIVER FAT PRODUCED BY ANTERIOR PITUITARY EXTRACTS

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The administration of anterior pituitary extracts may modify the physiological responses of the organism to insulin and produce alterations in the insulin-secreting processes. These effects have been described frequently in original papers and in reviews by Houssay (1942) and others.

The effects of insulin on the reactions produced by anterior pituitary extracts are in general not so clear or well known. Mirsky (1936b) found that the ketonemia produced by anterior pituitary extracts in rabbits was inhibited by insulin. Since evisceration completely suppressed the ketonemia produced by the anterior pituitary extracts (Mirsky, 1936a) it was indicated that the liver was the site of this antagonism. Certain anterior pituitary extracts produce a great mobilisation of fat and accumulation of fat in the livers of fasting animals (Best and Campbell, 1936, 1938). The effects of insulin on this response to the anterior pituitary have now been investigated.

METHODS. The total fatty acids plus the unsaponifiable fats were determined by the method of Leathes and Raper (1925). The anterior pituitary extract (A.P.P.) has been described (Best and Campbell 1936, 1938). The "ordinary" insulin and the protamine-zinc insulin were supplied by the Connaught Laboratories. "Control" injections of equivalent volumes of saline were given in lieu of extract or insulin. The subcutaneous injections of anterior pituitary extract and insulin were given in different sites to obviate direct interference of one with the other.

EXPERIMENTAL RESULTS. *Experiments with guinea pigs.* It has been shown by Best and Campbell (1938) that in guinea pigs fat accumulates in the liver during a fast and that a much greater accumulation of fat occurs after the injection of anterior pituitary extract. The effect of insulin on this response is shown in table 1. In the first experiment (groups a to d) the animals were given protamine-zinc insulin, to give a prolonged effect, immediately following the injection of anterior pituitary extract and the fasting period was begun at this time. After 10 hours a second injection of protamine-zinc insulin was given and 14 hours later the liver fat was determined. In two animals signs of insulin reactions were noted.

The anterior pituitary extract increased the liver fat from the control level of 202 mgm. per 100 grams' body weight to 720 mgm. and the insulin reduced this response to 410 mgm. An incomplete inhibition of the response therefore occurred.

In the second experiment (groups e to h) the animals were fasted for 16 hours prior to the injections and the dosage of protamine insulin was increased to near

maximal limits. Decided signs of insulin overdosage were noted (weakness and prostration). This heavy dosage of insulin inhibited the response to the anterior pituitary extract but again the inhibition was not complete. (The liver fat in the control group was 282 mgm. per 100 grams body weight; with anterior pituitary extract it was 886 mgm. and with the extract plus insulin, 802 mgm.) There was no significant effect of insulin on the increase in liver fat due to fasting alone.

TABLE 1

Female guinea pigs

Group a injections: 46 mgm. A.P.P. per kgm. at 0 hr.; 0.5 and 0.8 unit protamine-zinc insulin per kgm. at 0 and 10 hours, respectively.

Group b injections: 46 mgm. A.P.P. per kgm. at 0 hr.

Group c injections: 0.5 and 0.8 unit protamine-zinc insulin per kgm. at 0 and 10 hours respectively.

Group d injections: saline.

Fasted during the experimental period (24 hrs.)

Group e injections: 50 mgm. A.P.P. per kgm. at 1 hr.; 5 units protamine-zinc insulin per kgm. at 0 hr.

Group f injections: 50 mgm. A.P.P. per kgm. at 1 hr.

Group g injections: 5 units protamine-zinc insulin per kgm. at 0 hr.

Group h injections: saline at 0 hr.

Fasted for 16 hours previous to, and during the experimental period of 24 hours.

GROUPS	NUMBER OF ANIMALS	AVERAGE INITIAL WEIGHT	AVERAGE LOSS IN WEIGHT	LIVER WEIGHT PER 100 GRAMS	LIVER FAT PER 100 GRAMS	LIVER FAT
		<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>mgm.</i>	<i>per cent</i>
a	8	322	37	4.21	410	9.75
b	8	324	34	4.66	720	15.4
c	8	323	40	3.68	189	5.13
d	8	323	34	3.52	202	5.74
e	8	281	27	4.79	802	16.7
f	8	283	28	4.75	886	18.7
g	7	293	35	3.70	316	8.55
h	7	325	30	3.67	282	7.69

Experiment with rats. Although a considerable increase in liver fat can be produced in rats by the administration of anterior pituitary extract (Best and Campbell, 1938), they are relatively resistant in this respect when compared to guinea pigs and mice. McIntyre and Burke (1937) reported that rats could withstand massive doses of insulin. The strain of rats used in our experiments, however, could not tolerate the doses of insulin mentioned by these authors. The doses which could be tolerated were of the order mentioned by Bainbridge (1925), i.e., about 12 units of insulin per kgm. of body weight produced convulsions. The doses of insulin given in the experiment shown in table 2 were sufficient to produce signs of weakness and prostration in most of the animals both in the morning and evening of the day of injection. The insulin produced a slight

decrease in the amount of fat found in the liver following the injection of the anterior pituitary extract. The control liver fat level was 134 mgm. per 100 grams' body weight; 24 hours after the anterior pituitary injection the level was 575 mgm. and after anterior pituitary and insulin injections the level was 525 mgm. The experiment was continued for another 24 hours by giving other animals another injection of anterior pituitary extract (at 24 hrs.) and two injections of 10 and 6 units of insulin per kgm. at 24 and 36 hours from the start of the experiment. The heavy insulin dosage caused the death of two rats and signs of overdosage in others. At the end of the experiment the liver fat was 498 mgm. per 100 grams' body weight in the group given insulin with the anterior pituitary extract and 602 mgm. in the group given the anterior pituitary extract alone.

TABLE 2

Female rats

Group a injections: 150 mgm. A.P.P. per rat at 0 hour and 10 and 12 units insulin per kgm. at 0 hr. and 5 hrs. respectively.

Group b injections: 150 mgm. A.P.P. per rat at 0 hour.

Group c injections: 3.0 ml. saline at 0 hour.

Fasted 24 hours previous to the first injection. The fast was continued after injection.

GROUPS	NUMBER OF RATS	AVERAGE INITIAL WEIGHT	AVERAGE WEIGHT LOSS	LIVER WEIGHT PER 100 GRAMS RAT	LIVER FAT PER 100 GRAMS RAT	LIVER FAT
		<i>grams</i>	<i>grams</i>	<i>grams</i>	<i>mgm.</i>	<i>per cent</i>
a	5	193	11	3.74	525	14.1
b	5	196	7	3.58	575	16.1
c	5	169	14	3.07	134	4.4

In rats, therefore, as in guinea pigs, insulin produced a slight decrease in the infiltration of fat in the liver due to the administration of the anterior pituitary extract.

Experiments with mice. Mice are particularly suitable experimental animals for the study of the effect of insulin on the "liver fat" response since *a*, they are sensitive to the anterior pituitary extract; *b*, a relatively short experimental period is required during which they can be kept under the influence of insulin by repeated injections, and *c*, the responses to graded doses of anterior pituitary extract are well established (Campbell, 1938) so that small, minimal doses of extract can be given and the effect of insulin on the responses to these can be determined. In the experiment shown in table 3 the dose of anterior pituitary extract was just sufficient to produce a significant increase in liver fat in 7 hours. The insulin dosage was adjusted in groups *a*, *b* and *c* to produce signs of weakness and prostration. In groups *d*, *e* and *f* the effects of insulin were more severe as 5 of the 25 animals succumbed and 8 of the remaining 20 convulsed.

In the mice given anterior pituitary extract, the insulin produced a decrease in the per cent of fat in the liver to the control (fasting) level (groups *a*, *b* and *c*). In this experiment, however, the liver fat per 100 grams' body weight in the animals given anterior pituitary extract plus insulin was slightly above the control

level. This is considered to be a more reliable index than the per cent of fat in the liver since after the administration of the extract a definite increase in liver weight occurs (Best and Campbell, 1938). The increase in liver weight is due to increases in the water, fat, protein and non-protein nitrogenous constituents of the organ. In groups d, e and f insulin treatment caused a decrease in the amount of fat found in the liver following the administration of the anterior pituitary extract. Despite the heavy insulin dosage the inhibition was not complete. In the control group the liver fat was 359 mgm. per 100 grams' body weight; in the group given anterior pituitary extract the value was 727 mgm. and in the group given extract plus insulin the value was 541 mgm.

TABLE 3
Female mice

Group a injections: 1.0 mgm. A.P.P. per mouse at 0 hour; 0.05 unit insulin per mouse at 0, 2.5, 4.75 and 6.25 hours.

Group b injections: 1.0 mgm. A.P.P. per mouse at 0 hour.

Group c injections: 0.4 ml. saline at 0 hour.

Group d injections: 2.0 mgm. A.P.P. per mouse at 0 hour; 0.05, 0.025, and 0.0025 unit insulin per mouse at 0, 2 and 4.75 hours respectively.

Group e injections: 2.0 mgm. A.P.P. per mouse at 0 hour.

Group f injections: saline.

Fasted during the experimental period (7 hrs.)

GROUPS	NUMBER OF MICE	AVERAGE FINAL WEIGHT	LIVER WEIGHT PER 100 GRAMS	LIVER FAT PER 100 GRAMS	LIVER FAT
		<i>grams</i>	<i>mgm.</i>	<i>mgm.</i>	<i>per cent</i>
a	25	28	5.30	389	7.34
b	25	27	5.09	467	9.18
c	25	28	4.67	362	7.73
d	20	19	6.15	541	8.80
e	25	18	5.78	727	12.6
f	24	18	5.18	359	6.95

DISCUSSION. In the species studied, insulin produced a decrease in the amount of fat found in the liver in response to the administration of anterior pituitary extract. The inhibition of the response was however not complete even with small doses of anterior pituitary extract and large doses of insulin.

Wertheimer (1926) found that when insulin was given to fasting phlorizinised dogs the fat contents of the livers were reduced. The insulin also reduced the lipemia, glycosuria and ketonuria produced by the phlorizin administration. It was concluded, since the lipemia was reduced, that the insulin had acted peripherally and had prevented the mobilisation of fat to the liver, thus preventing the increase in liver fat and the production of ketones. Raper and Smith (1926) found no evidence that insulin influenced the production of ketone bodies by the liver perfused with blood. The production of ketones was found to be directly proportional to the fat content of the organ and inversely proportional to the glycogen content. The findings of Mirsky indicate that the site of the action of

insulin in suppressing ketosis is the liver and that the suppression can be most effective. In normal rabbits an anterior pituitary extract produced ketonemia which was completely abolished by hepatectomy and suppressed by insulin. In the present work, fat mobilisation, which is made evident by the loss of body fat and the gain in liver fat (Best and Campbell, 1936, 1938; Barrett, Best and Ridout, 1938), was inhibited less by insulin than was the metabolism of fats in relation to ketosis as observed by Mirsky. It was found by Mirsky, Grayman and Nelson (1942) that the increase in blood lipids which occurred in fasting ducks given diethylstilbestrol was not accompanied by ketosis. This indicates that the availability of fats and their oxidation in the liver may be independent and that an increase in the former does not necessarily influence the latter.

The administration of the anterior pituitary extract used, in addition to mobilising fat, has the effect of increasing liver glycogen over the fasting "control" level (Campbell, 1938). It, therefore, has the carbohydrate-sparing effect found by Russell and others, and probably decreases the utilisation of carbohydrate while increasing fat utilisation. Carbohydrate given "per os" decreases the amount of fat found in the liver in response to the administration of the anterior pituitary extract (Campbell). This indicates that the carbohydrate administered to fasting animals decreases the amount of fat mobilised from the depots either by a peripheral action of the increased blood sugar on the depots and/or by an effect of increased carbohydrate utilisation. The insulin may act either by stimulating the utilisation of some of the small carbohydrate reserves of the fasting animals or by increasing the efficiency of utilisation of carbohydrate formed by gluconeogenesis, or by a combination of these mechanisms, thus decreasing the fat mobilisation in response to the administration of the anterior pituitary extract.

SUMMARY

In fasting rats, mice and guinea pigs, the increase in liver fat which occurs in response to the administration of an anterior pituitary extract is partially inhibited by insulin.

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REFERENCES

- BAINBRIDGE, H. W. *J. Physiol.* **60**: 293, 1925.
BARRETT, H. M., C. H. BEST AND J. H. RIDOUT. *J. Physiol.* **93**: 367, 1938.
BEST, C. H. AND J. CAMPBELL. *J. Physiol.* **86**: 190, 1936.
J. Physiol. **92**: 91, 1938.
CAMPBELL, J. *Endocrinology* **23**: 692, 1938.
HOUSSAY, B. A. *Endocrinology* **30**: 884, 1942.
LEATHES, J. B., AND H. S. RAPER. *The fats*. London: Longmans, 1925.
MCINTYRE, A. R. AND J. C. BURKE. *This Journal* **119**: 364, 1937.

MIRSKY, I. A. This Journal **115**: 424, 1936a.

Ibid. **116**: 322, 1936b.

MIRSKY, I. A., I. GRAYMAN AND N. NELSON. Proc. Soc. Exper. Biol. and Med. **51**: 363, 1942.

RAPER, H. S. AND E. C. SMITH. J. Physiol. **62**: 17, 1926.

RUSSELL, J. A. Proc. Soc. Exper. Biol. and Med. **37**: 31, 1937.

Endocrinology **22**: 80, 1938.

This Journal **140**: 98, 1943.

WERTHEIMER, E. Pfüger's Arch. **213**: 280, 1926.

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